



PATENT

Attorney Docket: 04520/016002

5  
AND METHODS OF THEIR USE AND PREPARATION

MAR 17 2000  
TECH CENTER 1600/2900

Background

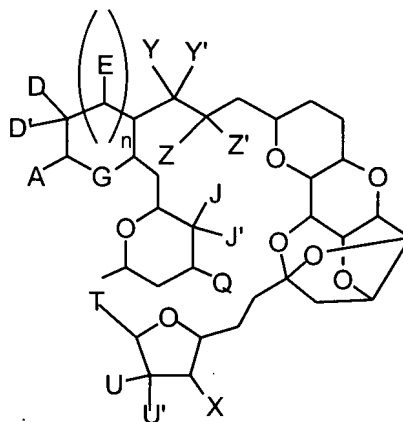
10 The invention relates to pharmaceutically active macrolides. Halichondrin B is a potent anticancer agent originally isolated from the marine sponge *Halichondria okadai*, and subsequently found in *Axinella sp.*, *Phakellia carteri*, and *Lissondendryx sp.*.

15 A total synthesis of Halichondrin B was published in 1992 (Aicher, T.D. et al., *J. Am. Chem. Soc.* 114:3162-3164). Halichondrin B has demonstrated *in vitro* inhibition of tubulin polymerization, microtubule assembly, beta<sup>S</sup>-tubulin crosslinking, GTP and vinblastine binding to tubulin, and tubulin-dependent GTP hydrolysis and has shown *in vitro* and *in vivo* anti-cancer properties.

Summary of the Invention

20 The invention provides halichondrin analogs having pharmaceutical activity, such as anticancer or antimitotic (mitosis-blocking) activity. These compounds are substantially smaller than halichondrin B. The invention features a compound having the formula (I):

tw30



**Formula (I)**

5 In formula (I), A is a  $C_{1-6}$  saturated or  $C_{2-6}$  unsaturated hydrocarbon skeleton, the skeleton being unsubstituted or having between 1 and 13 substituents, preferably between 1 and 10 substituents, e.g., at least one substituent selected from cyano, halo, azido,  $Q_1$ , and oxo. Each  $Q_1$  is independently selected from  $OR_1$ ,  $SR_1$ ,  $SO_2R_1$ ,  $OSO_2R_1$ ,  $NR_2R_1$ ,  $NR_2(CO)R_1$ ,  $NR_2(CO)(CO)R_1$ ,  $NR_4(CO)NR_2R_1$ ,  $NR_2(CO)OR_1$ ,  $(CO)OR_1$ ,  $O(CO)R_1$ ,  $(CO)NR_2R_1$ , and  $O(CO)NR_2R_1$ . The  
10 number of substituents can be, for example, between 1 and 6, 1 and 8, 2 and 5, or 1 and 4. Throughout the disclosure, numerical ranges are understood to be inclusive.

Each of  $R_1$ ,  $R_2$ ,  $R_4$ ,  $R_5$ , and  $R_6$  is independently selected from H,  $C_{1-6}$  alkyl,  $C_{1-6}$  haloalkyl,  $C_{1-6}$  hydroxyalkyl,  $C_{1-6}$  aminoalkyl,  $C_{6-10}$  aryl,  $C_{6-10}$  haloaryl (e.g., p-fluorophenyl or p-chlorophenyl),  $C_{6-10}$  hydroxyaryl,  $C_{1-4}$  alkoxy- $C_6$  aryl (e.g., p-methoxyphenyl, 3,4,5-  
15 trimethoxyphenyl, p-ethoxyphenyl, or 3,5-diethoxyphenyl),  $C_{6-10}$  aryl- $C_{1-6}$  alkyl (e.g., benzyl or phenethyl),  $C_{1-6}$  alkyl- $C_{6-10}$  aryl,  $C_{6-10}$  haloaryl- $C_{1-6}$  alkyl,  $C_{1-6}$  alkyl- $C_{6-10}$  haloaryl,  $(C_{1-3}$  alkoxy- $C_6$  aryl)- $C_{1-3}$  alkyl,  $C_{2-9}$  heterocyclic radical,  $C_{2-9}$  heterocyclic radical- $C_{1-6}$  alkyl,  $C_{2-9}$  heteroaryl, and  $C_{2-9}$  heteroaryl- $C_{1-6}$  alkyl. There may be more than one  $R_1$ , for example, if A is substituted with two different alkoxy ( $OR_1$ ) groups such as butoxy and 2-aminoethoxy.

Examples of A include 2,3-dihydroxypropyl, 2-hydroxyethyl, 3-hydroxy-4-perfluorobutyl, 2,4,5-trihydroxypentyl, 3-amino-2-hydroxypropyl, 1,2-dihydroxyethyl, 2,3-dihydroxy-4-perfluorobutyl, 3-cyano-2-hydroxypropyl, 2-amino-1-hydroxyethyl, 3-azido-2-hydroxypropyl, 3,3-difluoro-2,4-dihydroxybutyl, 2,4-dihydroxybutyl, 2-hydroxy-2(p-fluorophenyl)-ethyl, -CH<sub>2</sub>(CO)(substituted or unsubstituted aryl), -CH<sub>2</sub>(CO)(alkyl or substituted alkyl, such as haloalkyl or hydroxyalkyl) and 3,3-difluoro-2-hydroxypent-4-enyl.

Examples of Q<sub>1</sub> include -NH(CO)(CO)-(heterocyclic radical or heteroaryl), -OSO<sub>2</sub>-(aryl or substituted aryl), -O(CO)NH-(aryl or substituted aryl), aminoalkyl, hydroxyalkyl, -NH(CO)(CO)-(aryl or substituted aryl), -NH(CO)(alkyl)(heteroaryl or heterocyclic radical), O(substituted or unsubstituted alkyl)(substituted or unsubstituted aryl), and -NH(CO)(alkyl)(aryl or substituted aryl).

Each of D and D' is independently selected from R<sub>3</sub> and OR<sub>3</sub>, wherein R<sub>3</sub> is H, C<sub>1-3</sub> alkyl, or C<sub>1-3</sub> haloalkyl. Examples of D and D' are methoxy, methyl, ethoxy, and ethyl. In some embodiments, one of D and D' is H.

The value for n is 1 or preferably 0, thereby forming either a six-membered or five-membered ring. This ring can be unsubstituted or substituted, e.g., where E is R<sub>5</sub> or OR<sub>5</sub>, and can be a heterocyclic radical or a cycloalkyl, e.g. where G is S, CH<sub>2</sub>, NR<sub>6</sub>, or preferably O.

Each of J and J' is independently H, C<sub>1-6</sub> alkoxy, or C<sub>1-6</sub> alkyl; or J and J' taken together are =CH<sub>2</sub> or -O-(straight or branched C<sub>1-5</sub> alkylene or alkylidene)-O-, such as exocyclic methylenidene, isopropylidene, methylene, or ethylene. Q is C<sub>1-3</sub> alkyl, and is preferably methyl. T is ethylene or ethenylene, optionally substituted with (CO)OR<sub>7</sub>, where R<sub>7</sub> is H or C<sub>1-6</sub> alkyl. Each of U and U' is independently H, C<sub>1-6</sub> alkoxy, or C<sub>1-6</sub> alkyl; or U and U' taken together are =CH<sub>2</sub> or -O-(straight or branched C<sub>1-5</sub> alkylene or alkylidene)-O-. X is H or C<sub>1-6</sub> alkoxy. Each of Y and Y' is independently H or C<sub>1-6</sub> alkoxy; or Y and Y' taken together are =O, =CH<sub>2</sub>, or -O-(straight or branched C<sub>1-5</sub> alkylene or alkylidene)-O-. Each of Z and Z' is independently H or C<sub>1-6</sub> alkoxy; or Z and Z' taken together are =O, =CH<sub>2</sub>, or -O-(straight or branched C<sub>1-5</sub> alkylene or alkylidene)-O-.

The invention features compounds of sufficient stability to be suitable for pharmaceutical development. The invention also features pharmaceutically acceptable salts of disclosed compounds, disclosed novel synthetic intermediates, pharmaceutical compositions containing one or more disclosed compounds, methods of making the disclosed compounds or  
5 intermediates, and methods of using the disclosed compounds or compositions. Methods of use include methods for reversibly or irreversibly inhibiting mitosis in a cell, and for inhibiting cancer or tumor growth *in vitro*, *in vivo*, or in a patient. The invention also features methods for identifying an anti-mitotic or anti-cancer agent, such as a reversible or, preferably, an irreversible agent.

10

#### Brief Description of the Figures

*Handwritten: 1-8-01, fully not filed*  
FIG. 1 is a graph showing the percentage of cells which have completed mitosis and returned to the G<sub>1</sub> phase as a function of concentration of compound B1939 in a mitotic block  
15 assay. The minimum concentration required for complete mitotic block at 0 hour is 10 nM. The minimum concentration required for complete mitotic block at 10 hour (after washout) is also 10 nM. The reversibility ratio is therefore 1 for B1939. Superimposed upon this graph is a curve showing the percentage of viable cells at 5 days as a function of concentration of compound B1939. Viability drops to very low levels at the same concentration as the 10 hour mitotic block.

20 FIG. 2 is a graph showing the percentage of cells which have completed mitosis and returned to G<sub>1</sub> phase as a function of the concentration of compound B2042 in a mitotic block reversibility assay. The minimum concentration required for complete mitotic block at 0 hour is 3 nM. The minimum concentration required for complete mitotic block at 10 hour is 100 nM. The reversibility ratio for B2042 is therefore 33. Superimposed on this graph is a curve showing  
25 the percentage of viable cells at 5 days as a function of concentration of compound B2042. Viability drops to very low levels at the same concentration as the 10 hour mitotic block.

FIG. 3 is a graph showing the average tumor volume in microliters as a function of time (days) in a LOX melanoma xenograft growth inhibition assay. This illustrates the antitumor

activity of a compound of formula (I), compound B1939. Paclitaxel and a vehicle control were used.

FIG. 4 is a graph showing the average body weight per mouse as a function of time (days) in the assay described in FIG. 3.

FIG. 5 is a graph showing the average tumor volume in microliters as a function of time (days) in a COLO 205 human colon cancer xenograft growth inhibition assay, showing the antitumor activities of vinblastine and vincristine.

## Detailed Description of the Invention

- A. Definitions
- B. Halichondrin Analogs
- C. Synthesis of Halichondrin Analogs
- D. Pharmacological Activity
- E. Uses

### A. Definitions

The following terms are defined in part below and by their usage herein.

Hydrocarbon skeletons contain carbon and hydrogen atoms and may be linear, branched, or cyclic. Unsaturated hydrocarbons include one, two, three or more C-C double bonds ( $sp^2$ ) or C-C triple bonds ( $sp$ ). Examples of unsaturated hydrocarbon radicals include ethynyl, 2-propynyl, 1-propenyl, 2-butenyl, 1,3-butadienyl, 2-pentenyl, vinyl (ethenyl), allyl, and isopropenyl. Examples of bivalent unsaturated hydrocarbon radicals include alkenylenes and alkylidenes such as methylidyne, ethylidene, ethylidyne, vinylidene, and isopropylidene. In general, compounds of the invention have hydrocarbon skeletons ("A" in formula (I)) that are substituted, e.g., with hydroxy, amino, cyano, azido, heteroaryl, aryl, and other moieties

described herein. Hydrocarbon skeletons may have two geminal hydrogen atoms replaced with oxo, a bivalent carbonyl oxygen atom ( $=O$ ), or a ring-forming substituent, such as  $-O-$ (straight or branched alkylene or alkylidene)- $O-$  to form an acetal or ketal.

$C_{1-6}$  alkyl includes linear, branched, and cyclic hydrocarbons, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, sec-pentyl, neo-pentyl, tert-pentyl, cyclopentyl, hexyl, isohexyl, sec-hexyl, cyclohexyl, 2-methylpentyl, tert-hexyl, 2,3-dimethylbutyl, 3,3-dimethylbutyl, 1,3-dimethylbutyl, and 2,3-dimethyl but-2-yl. Alkoxy ( $-OR$ ), alkylthio ( $-SR$ ), and other alkyl-derived moieties (substituted, unsaturated, or bivalent) are analogous to alkyl groups ( $R$ ). Alkyl groups, and alkyl-derived groups such as the representative alkoxy, haloalkyl, hydroxyalkyl, alkenyl, alkylidene, and alkylene groups, can be  $C_{2-6}$ ,  $C_{3-6}$ ,  $C_{1-3}$ , or  $C_{2-4}$ .

Alkyls substituted with halo, hydroxy, amino, cyano, azido, and so on can have 1, 2, 3, 4, 5 or more substituents, which are independently selected (may or may not be the same) and may or may not be on the same carbon atom. For example, haloalkyls are alkyl groups with at least one substituent selected from fluoro, chloro, bromo, and iodo. Haloalkyls may have two or more halo substituents which may or may not be the same halogen and may or may not be on the same carbon atom. Examples include chloromethyl, periodomethyl, 3,3-dichloropropyl, 1,3-difluorobutyl, and 1-bromo-2-chloropropyl.

Heterocyclic radicals and heteroaryls include furyl, pyranal, isobenzofuranyl, chromenyl, xanthenyl, phenoxathienyl, 2H-pyrrolyl, pyrrolyl, imidazolyl (e.g., 1-, 2- or 4- imidazolyl), pyrazolyl, isothiazolyl, isoxazolyl, pyridyl (e.g., 1-, 2-, or 3- pyridyl), pyrazinyl, pyrimidinyl, pyridazinyl, indolizynyl, isoindolyl, 3H-indolyl, indolyl (e.g., 1-, 2-, or 3-indolyl), indazolyl, purinyl, 4H-quinolixynyl, isoquinolyl, quinolyl, phthalazinyl, naphthyridinyl, quinoxalynyl, quinazolylnyl, cinnolynyl, pteridinyl, pyrrolinyl, pyrrolidinyl, imidazolidinyl, pyrazolidinyl, pyrazolynyl, piperidyl, piperazinyl, indolynyl, isoindolynyl, and morpholynyl. Heterocyclic radicals and heteroaryls may be linked to the rest of the molecule at any position along the ring. Heterocyclic radicals and heteroaryls can be  $C_{2-9}$ , or smaller, such as  $C_{3-6}$ ,  $C_{2-5}$ , or  $C_{3-7}$ .

Aryl groups include phenyl, benzyl, naphthyl, tolyl, mesityl, xylyl, and cumenyl.

It is understood that "heterocyclic radical", "aryl", and "heteroaryl" include those having 1, 2, 3, 4, or more substituents independently selected from lower alkyl, lower alkoxy, amino, halo, cyano, nitro, azido, and hydroxyl. Heterocyclic radicals, heteroaryls, and aryls may also be bivalent substituents of hydrocarbon skeleton "A" in formula (I).

5

## B. Halichondrin Analogs

Referring to formula (I) in the Summary section, embodiments of the invention include compounds wherein n is 0; wherein each of D and D' is independently selected from R<sub>3</sub>, C<sub>1-3</sub> alkoxy, and C<sub>1-3</sub> haloalkyloxy; wherein R<sub>5</sub> is selected from H, C<sub>2-6</sub> alkyl, C<sub>1-6</sub> haloalkyl, C<sub>1-6</sub> hydroxyalkyl, C<sub>1-6</sub> aminoalkyl, C<sub>6-10</sub> aryl, C<sub>6-10</sub> haloaryl, C<sub>6-10</sub> hydroxyaryl, C<sub>1-3</sub> alkoxy-C<sub>6</sub> aryl, C<sub>6-10</sub> aryl-C<sub>1-6</sub> alkyl, C<sub>1-6</sub> alkyl-C<sub>6-10</sub> aryl, C<sub>6-10</sub> haloaryl-C<sub>1-6</sub> alkyl, C<sub>1-6</sub> alkyl-C<sub>6-10</sub> haloaryl, (C<sub>1-3</sub> alkoxy-C<sub>6</sub> aryl)-C<sub>1-3</sub> alkyl, C<sub>2,9</sub> heterocyclic radical, C<sub>2,9</sub> heterocyclic radical-C<sub>1-6</sub> alkyl, C<sub>2,9</sub> heteroaryl, and C<sub>2,9</sub> heteroaryl-C<sub>1-6</sub> alkyl; and combinations thereof.

Other embodiment includes compounds having one or more of the following characteristics: (a) wherein A is a C<sub>1-6</sub> saturated or C<sub>2-6</sub> unsaturated hydrocarbon skeleton, the skeleton having at least one substituent selected from cyano, halo, azido, Q<sub>1</sub>, and oxo; (b) each Q<sub>1</sub> is independently selected from OR<sub>1</sub>, SR<sub>1</sub>, SO<sub>2</sub>R<sub>1</sub>, OSO<sub>2</sub>R<sub>1</sub>, NR<sub>2</sub>R<sub>1</sub>, NR<sub>2</sub>(CO)R<sub>1</sub>, NR<sub>2</sub>(CO)R<sub>1</sub>, and O(CO)NR<sub>2</sub>R<sub>1</sub>; (c) Z and Z' taken together are =O or =CH<sub>2</sub>; (d) wherein each Q<sub>1</sub> is independently selected from OR<sub>1</sub>, SR<sub>1</sub>, SO<sub>2</sub>R<sub>1</sub>, OSO<sub>2</sub>R<sub>1</sub>, NH(CO)R<sub>1</sub>, NH(CO)(CO)R<sub>1</sub>, and O(CO)NHR<sub>1</sub>; (e) each R<sub>1</sub> is independently selected from C<sub>1-6</sub> alkyl, C<sub>1-6</sub> haloalkyl, C<sub>6</sub> aryl, C<sub>6</sub> haloaryl, C<sub>1-3</sub> alkoxy-C<sub>6</sub> aryl, C<sub>6</sub> aryl-C<sub>1-3</sub> alkyl, C<sub>1-3</sub> alkyl-C<sub>6</sub> aryl, C<sub>6</sub> haloaryl-C<sub>1-3</sub> alkyl, C<sub>1-3</sub> alkyl-C<sub>6</sub> haloaryl, (C<sub>1-3</sub> alkoxy-C<sub>6</sub> aryl)-C<sub>1-3</sub> alkyl, C<sub>2,9</sub> heterocyclic radical, C<sub>2,9</sub> heteroaryl, and C<sub>2,9</sub> heteroaryl-C<sub>1-6</sub> alkyl; (f) one of D and D' is methyl or methoxy and the other is H; (g) n is 0; (h) G is O; (i) J and J' taken together are =CH<sub>2</sub>; (j) Q is methyl; (k) T is ethylene; (l) U and U' taken together are =CH<sub>2</sub>; (m) X is H; (n) each of Y and Y' is H; and (o) Z and Z' taken together are =O. Examples of combinations are the combination of (h)-(m), the combination of (a) and (b), the combination of (f) and (h), and the combination of (h) and where one of D and D' is

methyl and the other is H. Two particularly preferred compounds are B1793 and B1939.

Another embodiment includes compounds wherein  $Q_1$  is independently selected from  $OR_1$ ,  $SR_1$ ,  $SO_2R_1$ , and  $OSO_2R_1$ ; and each  $R_1$  is independently selected from  $C_{1-6}$  alkyl,  $C_{1-6}$  haloalkyl,  $C_6$  aryl,  $C_6$  haloaryl,  $C_{1-3}$  alkoxy- $C_6$  aryl,  $C_6$  aryl- $C_{1-3}$  alkyl,  $C_{1-3}$  alkyl- $C_6$  aryl,  $C_6$

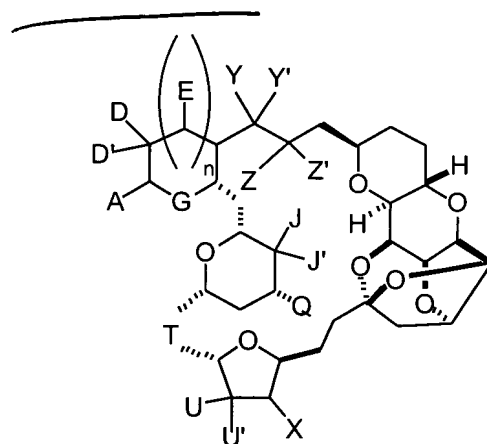
5 haloaryl- $C_{1-3}$  alkyl,  $C_{1-3}$  alkyl- $C_6$  haloaryl, and  $(C_{1-3}$  alkoxy- $C_6$  aryl)- $C_{1-3}$  alkyl. Other embodiments include compounds wherein: one of D and D' is alkyl or alkoxy, where n is 1; (f) as above, where n is 1; E is alkoxy, where n is 1; n is 0, where one of D and D' is hydroxy and the other is H; and (f) as above, where n is 1 and E is methyl.

The invention also features compounds wherein: (1) A has at least one substituent  
10 selected from hydroxyl, amino, azido, halo, and oxo; (2) A is a saturated hydrocarbon skeleton having at least one substituent selected from hydroxyl, amino, and azido (e.g., B1793, B1939, B2042, B1794, and B1922); (3) A has at least two substituents independently selected from hydroxyl, amino, and azido (e.g., B2090 and B2136); (4) A has at least two substituents independently selected from hydroxyl and amino (e.g., B2042 and B2090); (5) A has at least one  
15 hydroxyl substituent and at least one amino substituent (e.g., B1939 and B2136); (6) A has at least two hydroxyl substituents (e.g., B1793 and B1794); (7) A is a  $C_{2-4}$  hydrocarbon skeleton that is substituted (e.g., B2004, B2037, B1920, B2039, B2070, B2090, and B2043); (8) A is a  $C_3$  hydrocarbon skeleton that is substituted (e.g., B1793, B1920, B1984, B1988, B1939, B1940, B2014); (9) A has an (S)-hydroxyl alpha to the carbon atom linking A to the ring containing G  
20 (e.g., B1793, B1939 or B1920) or an (R)-hydroxyl (e.g. B2102, B2013, B2042); and (10) A is a  $C_{1-6}$  saturated hydrocarbon skeleton having at least one substituent selected from hydroxyl and cyano (e.g., B2013, B2037, B2102, B2086, and B2091). By (S)-hydroxyl is meant the configuration of the carbon atom having the hydroxyl group is (S). Embodiments of the invention also include compounds which have at least two substituents on the carbon atoms (1)  
25 alpha and gamma, (2) beta and gamma, or preferably (3) alpha and beta to the carbon atom linking A to the ring containing G. The alpha, beta, and gamma carbon atoms can have an (R) or (S) configuration

The invention further provides preferred compounds having the formula (1) -A, shown

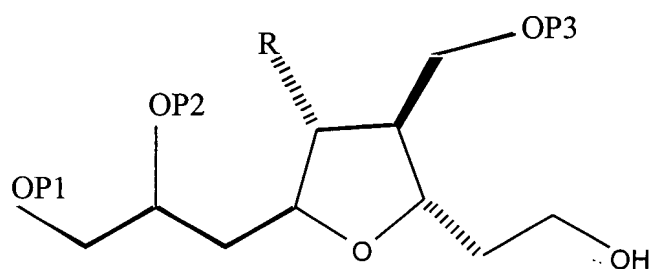


below, wherein the substituents are identical to those defined above.



Formula 1-A

5 The invention further features the following monosaccharide intermediate having formula (II):



Formula (II)

10 wherein R is methyl or methoxy, and each of P1, P2, and P3 is independently selected from H and primary alcohol protecting groups. Preferably, the diol sidechain is below the plane of the page and OP2 is above the plane of the page. Primary alcohol protecting groups include esters,  
15 ethers, silyl ethers, alkyl ethers, and alkoxyalkyl ethers.

Examples of esters include formates, acetates, carbonates, and sulfonates. Specific

examples include formate, benzoyl formate, chloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate, 4,4-(ethylenedithio)pentanoate, pivaloate, crotonate, 4-methoxy- crotonate, benzoate, p-phenylbenzoate, 2,4,6-trimethylbenzoate, carbonates such as methyl, 9-fluorenylmethyl, ethyl, 2,2,2-trichloroethyl, 2-(trimethylsilyl)ethyl, 2-(phenylsulfonyl)ethyl, vinyl, allyl, and p-nitrobenzyl.

Examples of silyl ethers include trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, triisopropylsilyl, and other trialkylsilyl ethers. Alkyl ethers include methyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, trityl, t-butyl, allyl, and allyloxycarbonyl ethers or derivatives. Alkoxyalkyl ethers include acetals such as methoxymethyl, methylthiomethyl, (2-methoxyethoxy)methyl, benzyloxymethyl, beta-(trimethylsilyl)ethoxymethyl, and tetrahydropyranyl ethers. Examples of benzyl ethers include p-methoxybenzyl (MPM), 3,4-dimethoxybenzyl, O-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, 2- and 4-picolyl. Preferably, each of P1 and P2 are TBS and P3 is MPM (see alcohol 19 below). In one aspect, formula (II) can be modified so the hydroxyethyl sidechain can also be a protected hydroxyl,  $-\text{CH}_2\text{CH}_2\text{O-P}_4$ , wherein P4 is independently selected from values for P1. A related intermediate is alcohol 17, where the hydroxyethyl sidechain is a hydroxymethyl sidechain. A corresponding hydroxypropyl sidechain, or aminoalkyl side chain, can be similarly prepared.

P1 and P2, taken together, can be a diol protecting group, such as cyclic acetals and ketals (methylene, ethylidene, benzylidenes, isopropylidene, cyclohexylidene, and cyclopentylidene), silylene derivatives such as di-t-butylsilylene and 1,1,3,3-tetra-isopropylidisiloxanylidene derivatives, cyclic carbonates, and cyclic boronates. Methods of adding and removing such hydroxyl protecting groups, and additional protecting groups, are well-known in the art and available, for example, in P.J. Kocienski, Protecting Groups, Thieme, 1994, and in T.W. Greene and P.G.M. Wuts, Protective Groups in Organic Synthesis, 2<sup>nd</sup> edition, John Wiley & Sons, 1992.

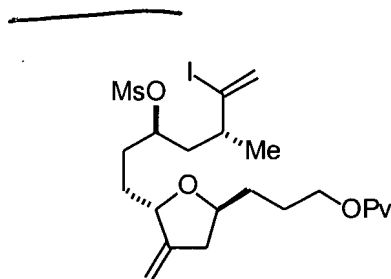
The following section provides representative syntheses of intermediates

of formula (II) and halichondrin analogs of formula (I).

### C. Synthesis of Halichondrin Analogs

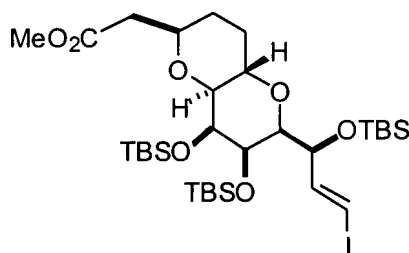
5 An overview is provided below, followed by synthetic schemes 1-16, and several detailed protocols.

Compounds of general formula 4 can be prepared by the route outlined in Scheme 1. Key fragment **F-2** exemplified by vinyl iodide compound **X2** can be prepared according to the procedure of Kishi, et al. (Total synthesis of halichondrin B and norhalichondrin B. Aicher, T. D.; Buszek, K. R.; Fang, F. G.; Forsyth, C. J.; Jung, S. H.; Kishi, Y.; Matelich, M. C.; Scola, . M.; Spero, D. M.; Yoon, S. K. *J. Am. Chem. Soc.* **1992**, *114*, 3162-4).



Vinyl Iodide **X2**

Toldo



**XF3**

Key fragment **F-3** can be obtained by DIBALH reduction of the corresponding methyl ester, **XF3**, prepared according to the procedure of Stamos, et al (Scheme 2). [Synthetic studies on halichondrins: a practical synthesis of the C.1-C.13 segment. Stamos, D. P.; Kishi, Y.

*Tetrahedron Lett.* **1996**, 37, 8643-8646]. Synthesis of key fragment **F-1** exemplified by compound **20** can be synthesized as described in Scheme 3 or Scheme 4.

Using **B1793** as a representative example, coupling of the three key fragments proceeded as outlined in Scheme 5: Nozaki-Hiyama-Kishi coupling of fragments **20** and **X2** followed by  
5 intramolecular Williamson ether formation furnished tetrahydropyran **B2318**. Protecting group modification as described in Scheme 5 or alternatively in Scheme 6 afforded primary iodide **B2313**. Halogen-metal exchange reaction and coupling with key fragment **F-3** furnished a mixture of diastereomeric alcohols **B2308**. Additional protecting group manipulation and oxidation followed by an intramolecular Nozaki-Hiyama-Kishi reaction afforded an intermediate,  
10 which when oxidized and treated with TBAF underwent intramolecular hetero-Michael ring closure. PPTs mediated acetal formation furnished **B1793**.

Aryl groups can be incorporated into the C32 sidechain (e.g. **B2043**) as exemplified in Scheme 7. Intermediate **B2318** was deprotected and the resulting diol oxidatively cleaved to the corresponding aldehyde. Treatment with a Grignard reagent (e.g. p-F-PhMgBr), separation of  
15 the resulting diastereomers and silylation furnished **204**, which was converted to final product in a manner similar to that described in Scheme 6.

Ether analogs can be prepared from **B1793** by treatment with an appropriate alkylating agent (e.g. Scheme 8). Similarly, sulfonates, esters, carbamates, etc. can be prepared from **B1793** by treatment with an activated carbonyl component. Oxidative diol cleavage and  
20 reduction or selective hydroxyl group oxidation could furnish derivatives such as **B2037** and **B1934**, respectively.

Alternatively, one or more hydroxyl groups could be converted to the corresponding amino groups with subsequent coupling with an activated carbonyl component (Scheme 9). Displacement of the sulfonyl intermediate (e.g. **B1920**) by carbon or heteroatom nucleophiles  
25 could also be readily accomplished (Scheme 10).

C31 methyl analogs can be prepared as outlined in Scheme 11. Indium mediated coupling of an allyl bromide ester with 2,3-O-(3-isopropylidene)-D-glyceraldehyde furnished lactone **103**. Hetero Michael addition, lactone reduction, Wittig coupling and intramolecular

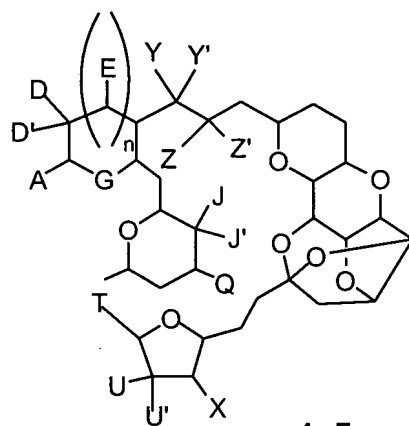
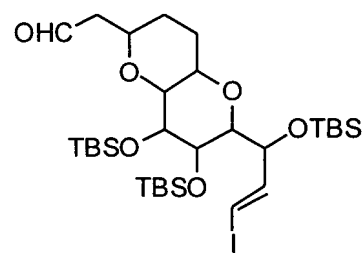
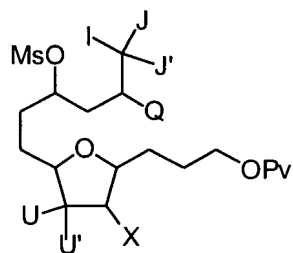
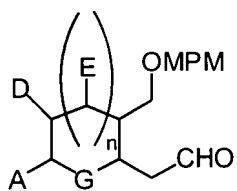
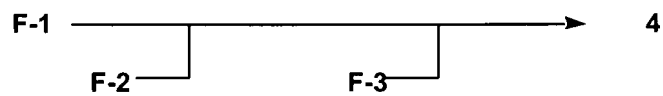
Michael addition furnished tetrahydrofuran **107**. Pummerer rearrangement, protecting group adjustment and DIBALH reduction furnished key fragment **1** (e.g., **114**), which was converted to final compound in a manner analogous to that described in Scheme 6.

5 Fluorine atoms could be introduced as described in Schemes 12 - 14. Beginning with the appropriate tetrahydrofuran intermediate, fluorinated key fragment **1** was obtained and carried to final compound in a manner analogous to that illustrated in Scheme 6.

Triol derivatives could be similarly prepared from the tetrahydrofuran intermediate. For example, as outlined in Scheme 15 allyltributylstannane addition to aldehyde **X32** furnished homoallylic alcohol **33** that was carried to final compound in a similar manner to that described  
10 in Scheme 6. These triols could be further modified as exemplified in Scheme 6.

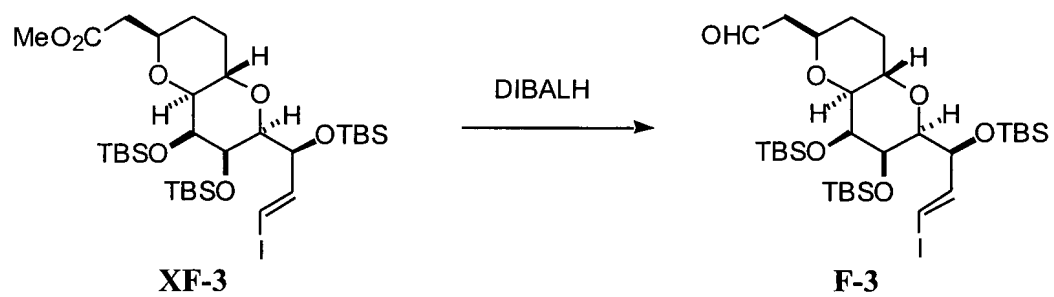
The 1,3 diol derivatives could be prepared from intermediates previously described. For example, **B2086** could be oxidatively cleaved and reduced to afford 1,3-diol **B2091** (Scheme 16).

Scheme 1

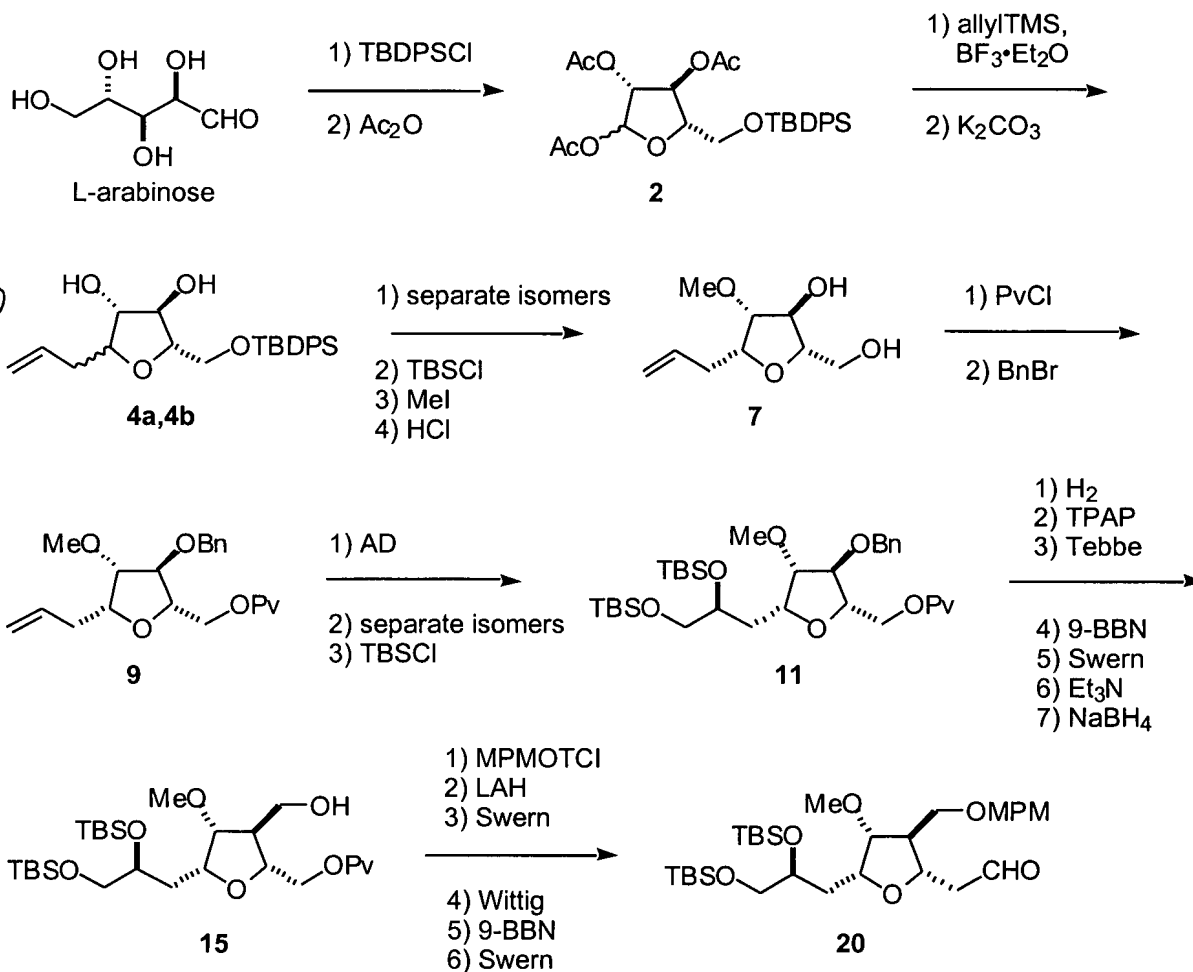


T0150

Scheme 2

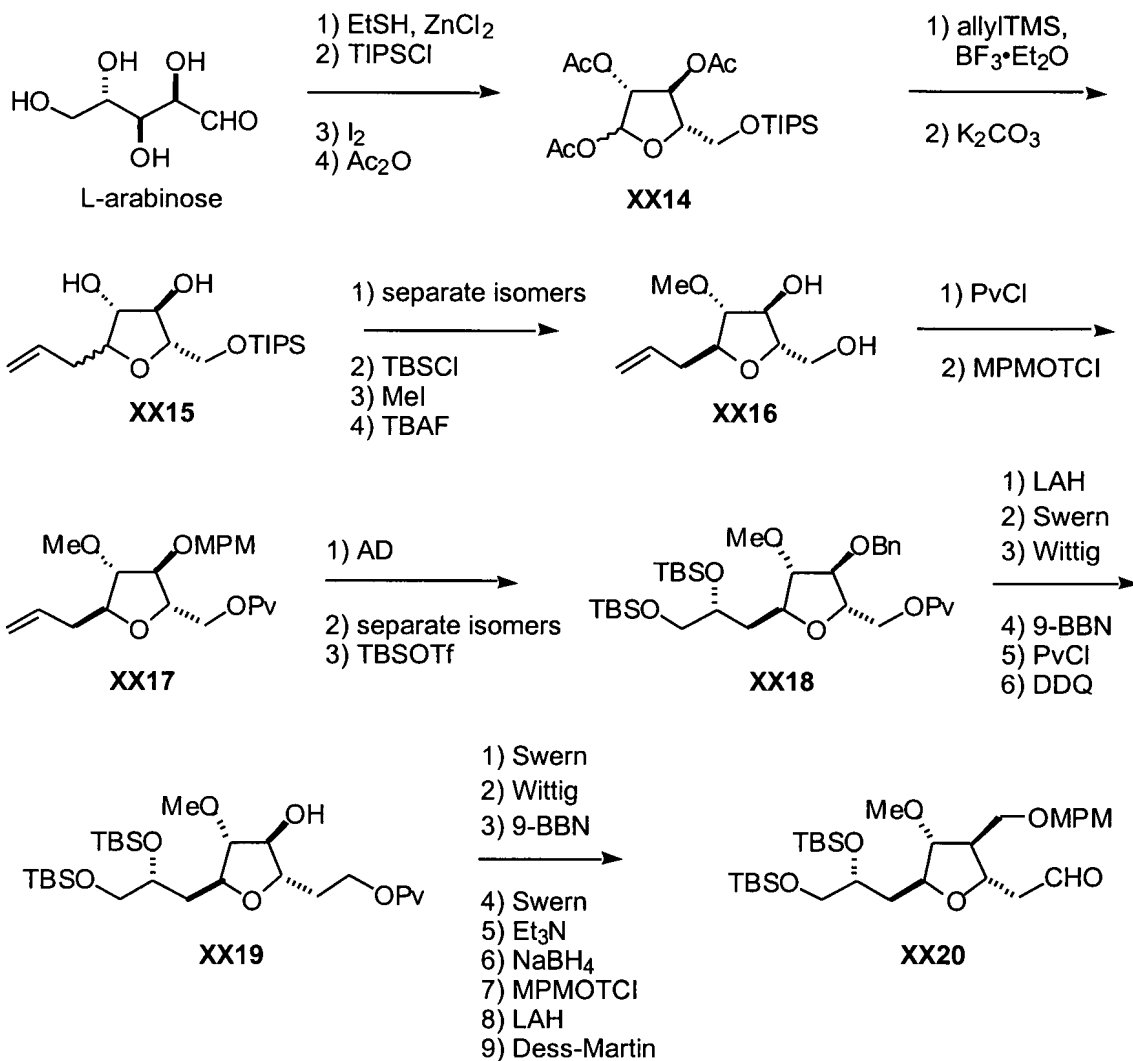


**Scheme 3**

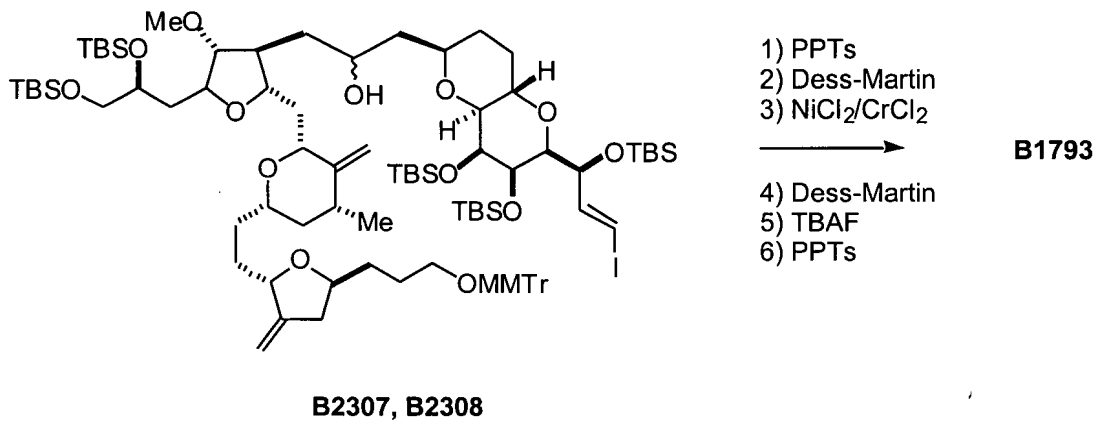
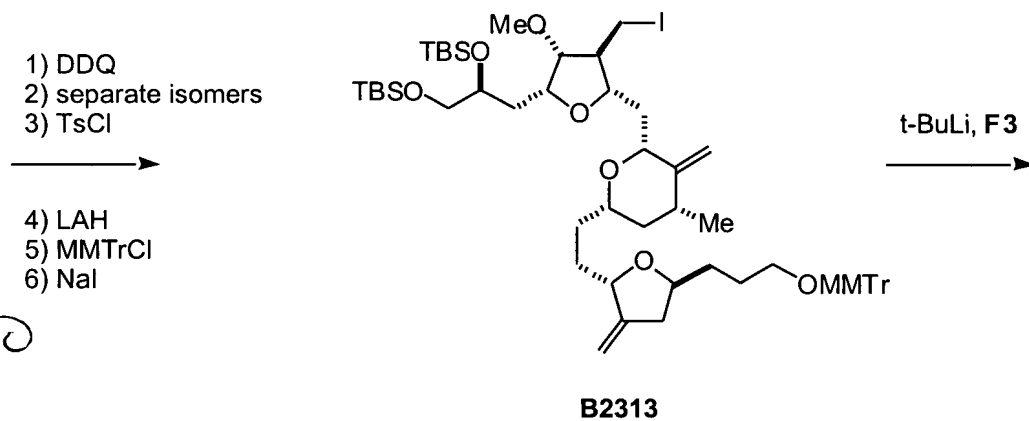
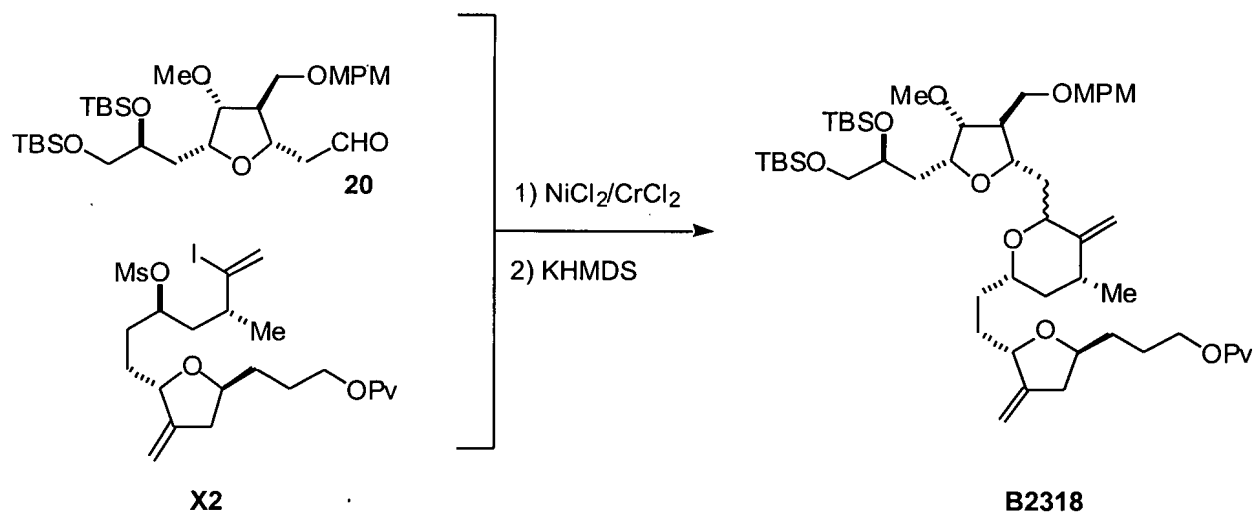




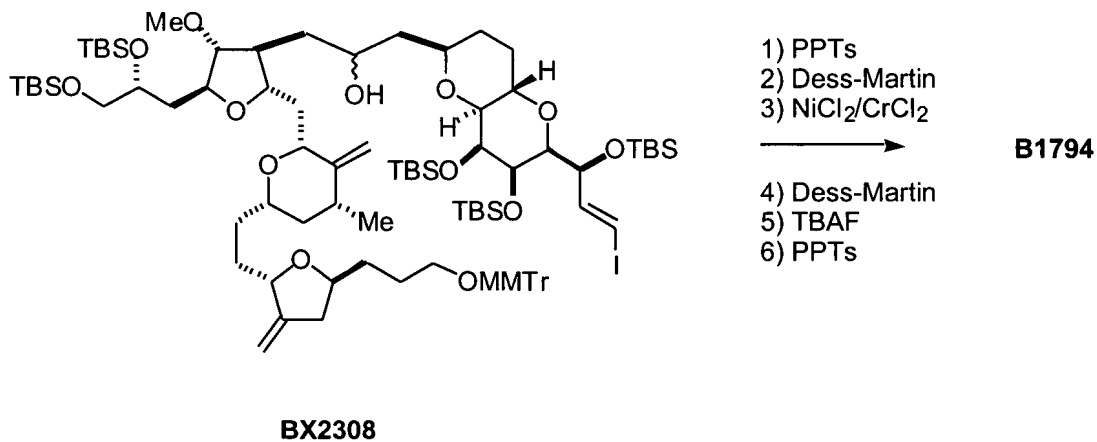
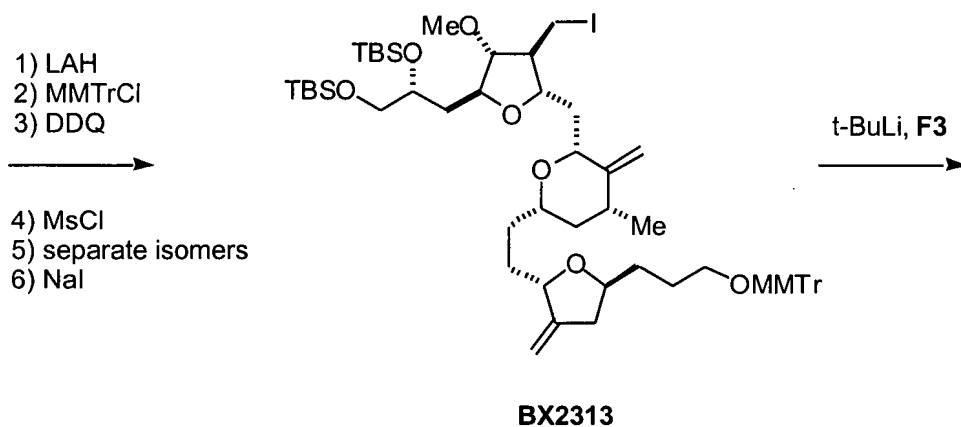
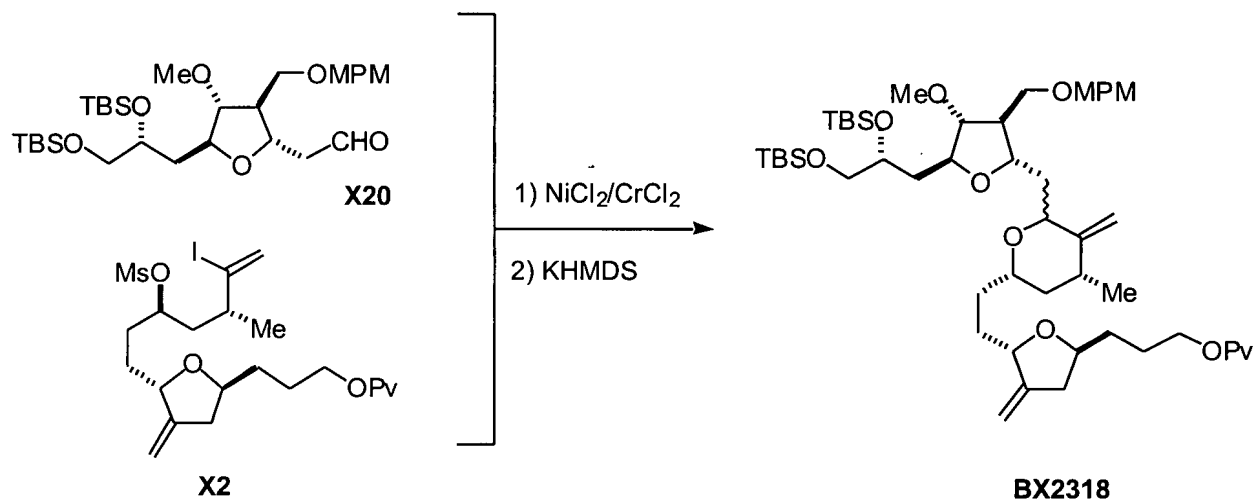
**Scheme 4**



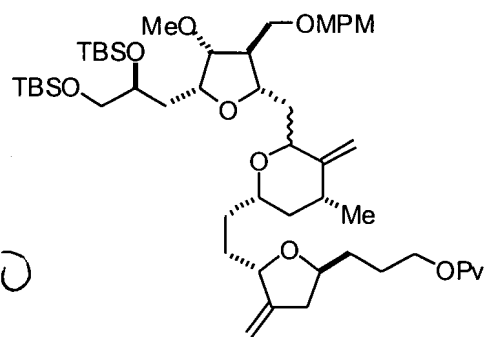
**Scheme 5**



Scheme 6

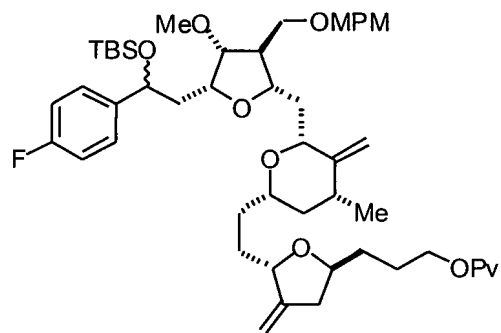


Scheme 7



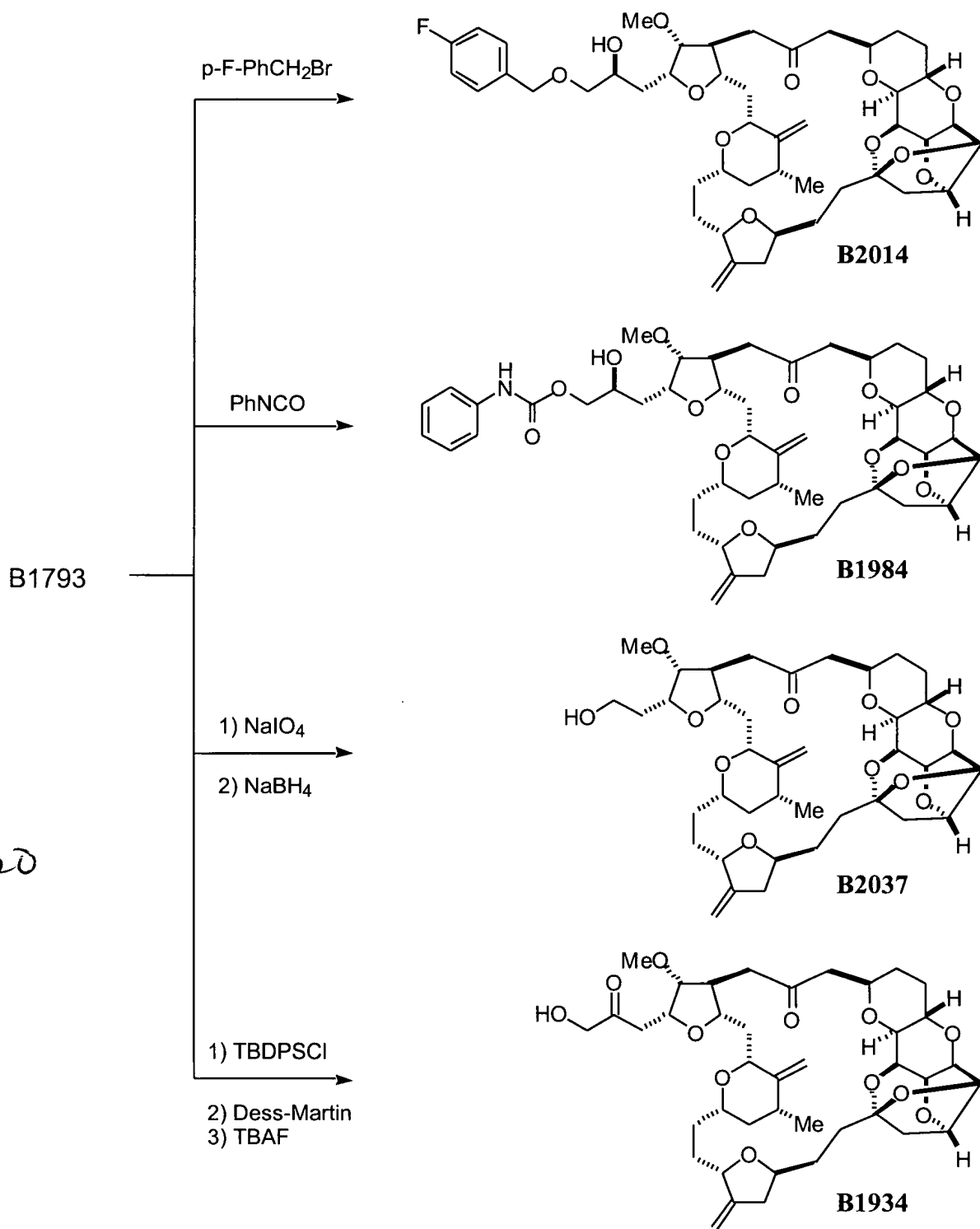
B2318

- 1) TBAF
- 2)  $\text{NaIO}_4$
- 3) p-F-PhMgBr
- 4) separate isomers
- 5) TBSOTf

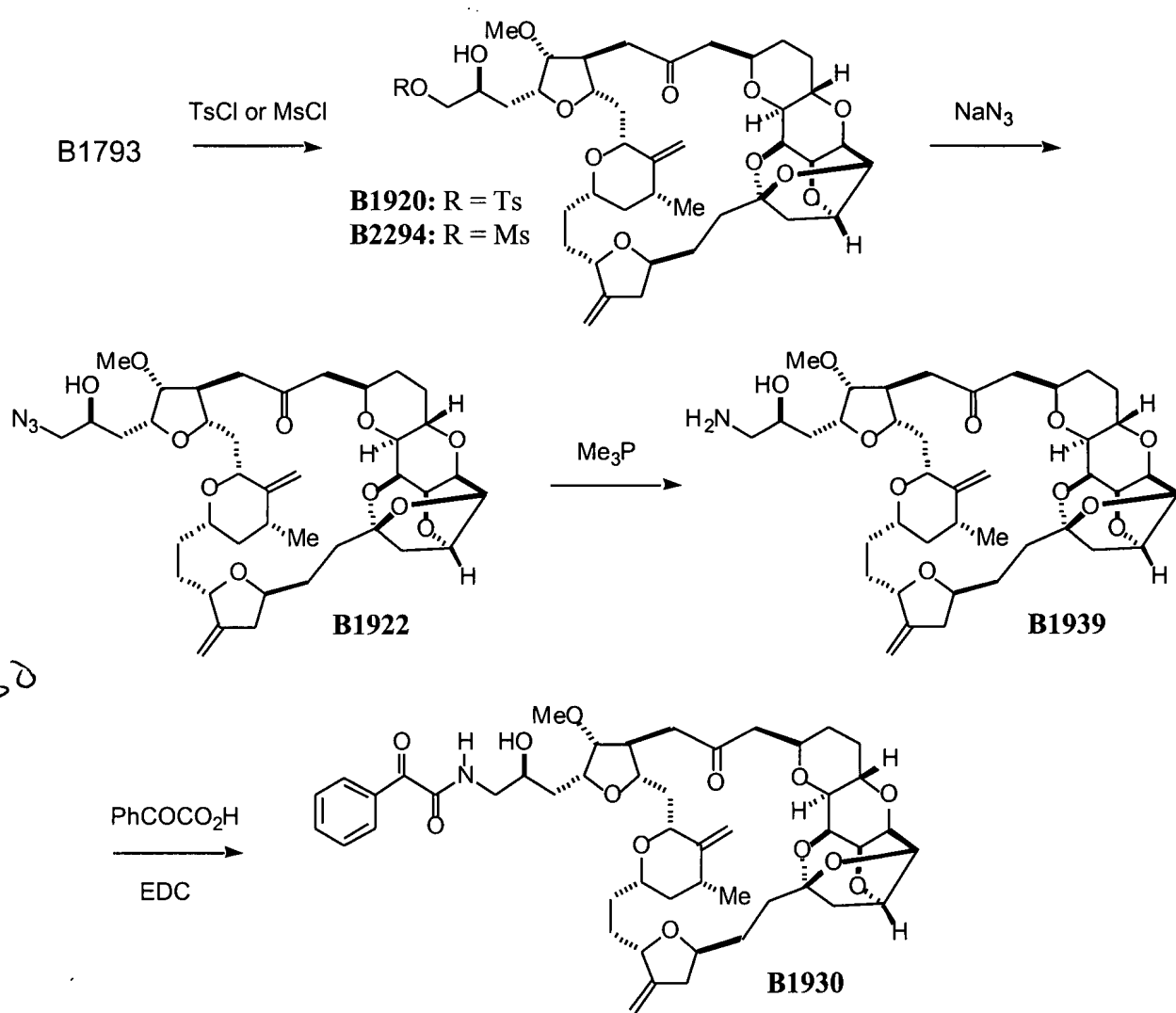


204

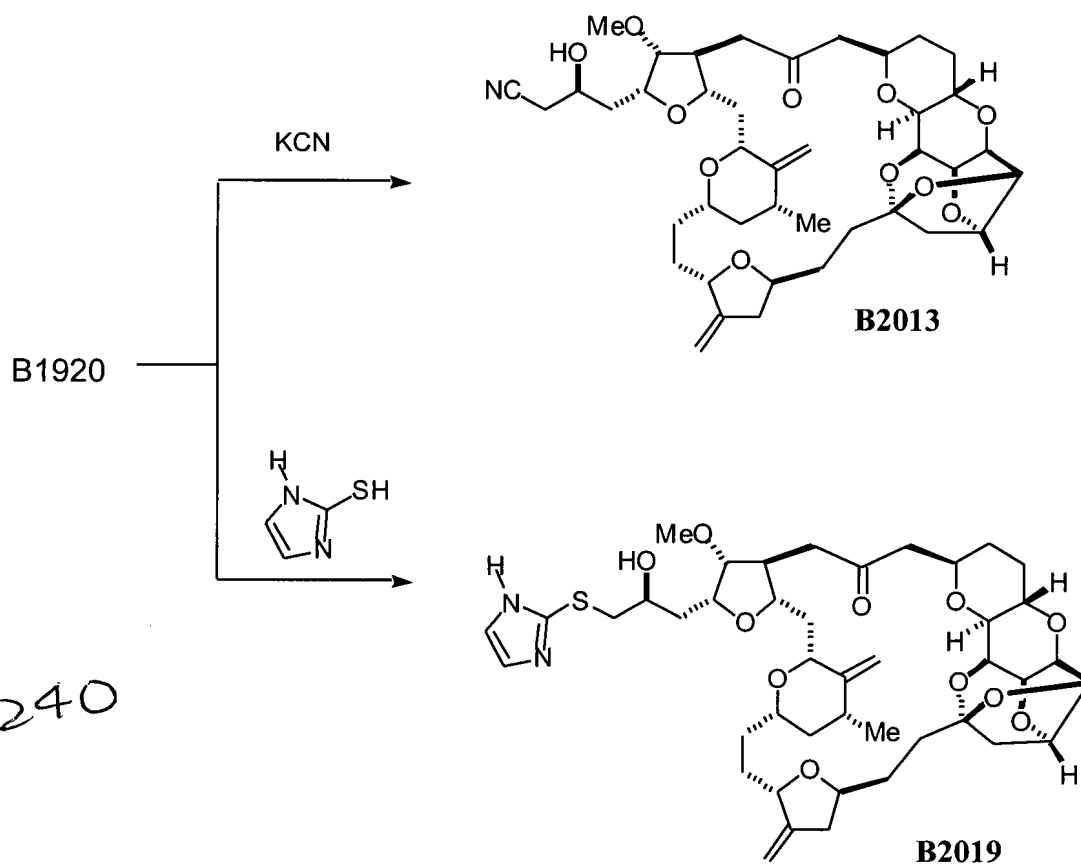
Scheme 8



Scheme 9

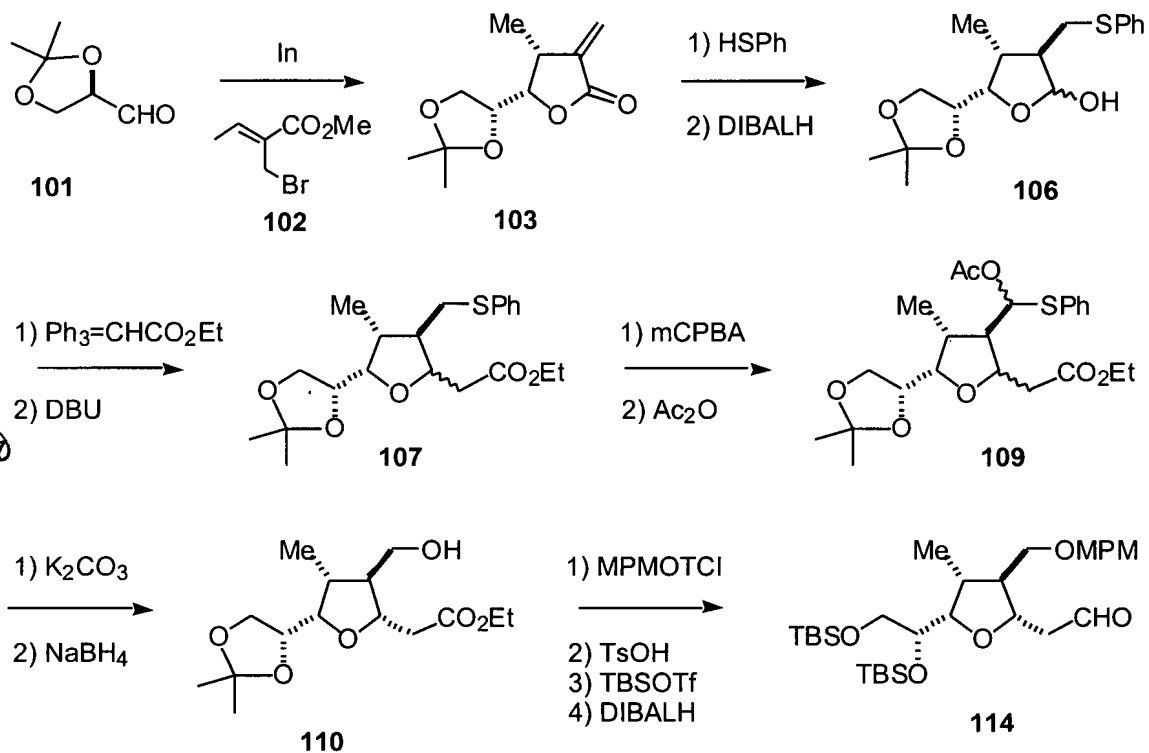


Scheme 10



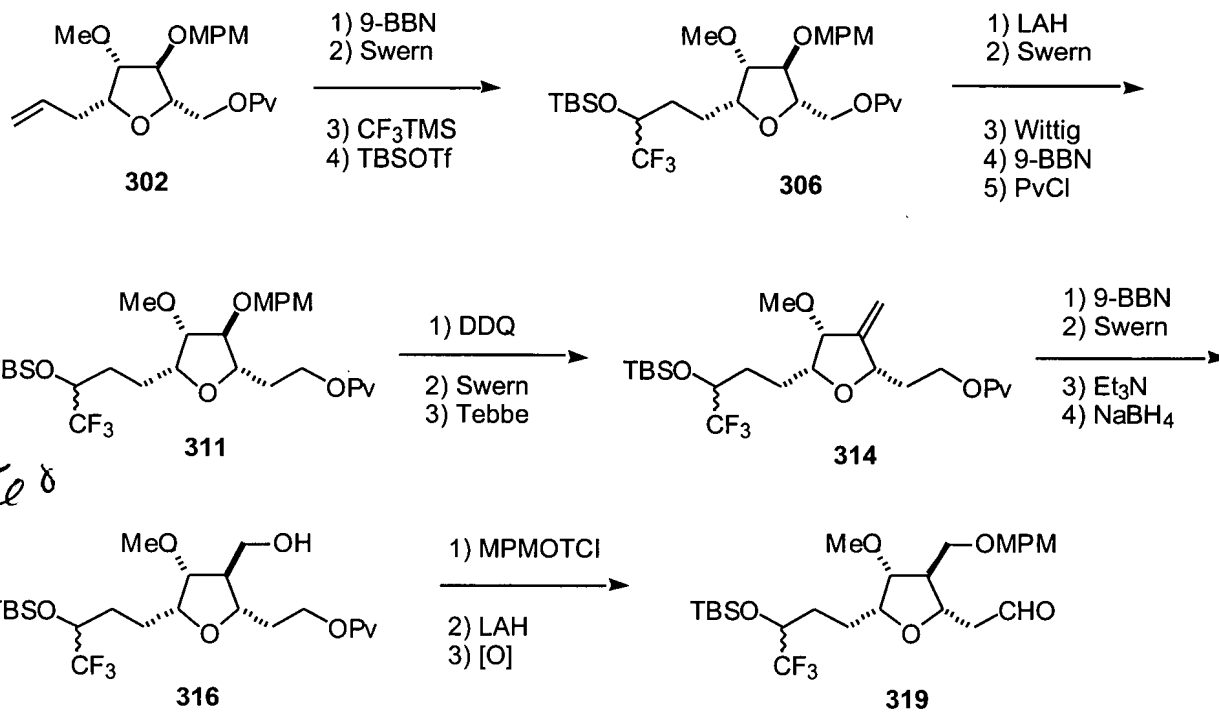
T 0240

Scheme 11

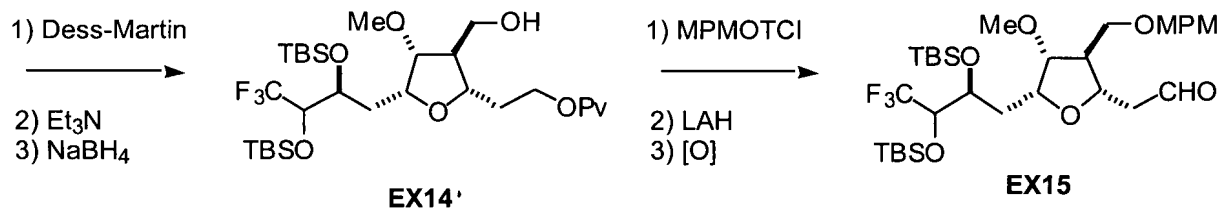
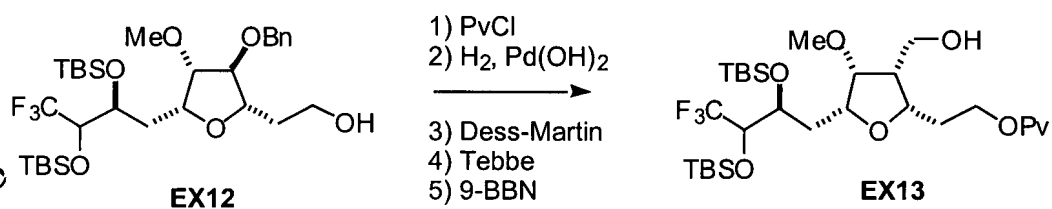




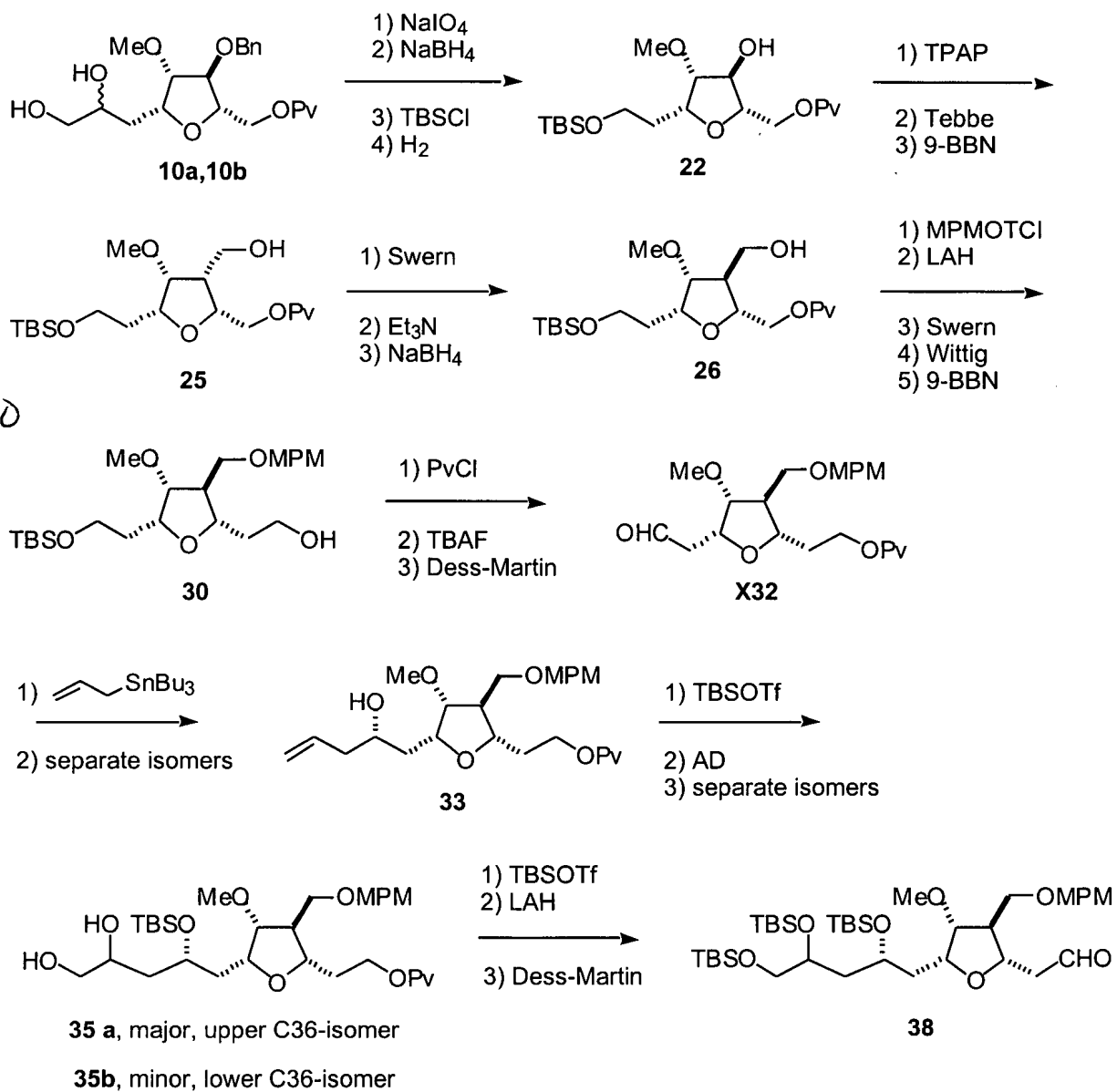
Scheme 12



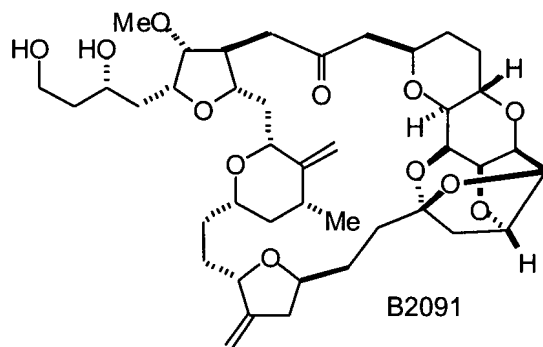
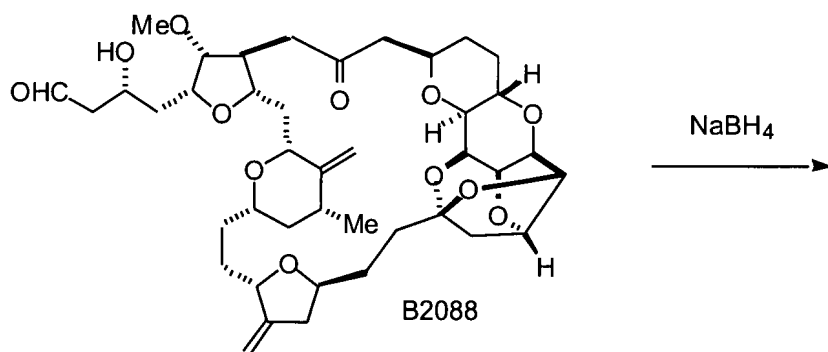
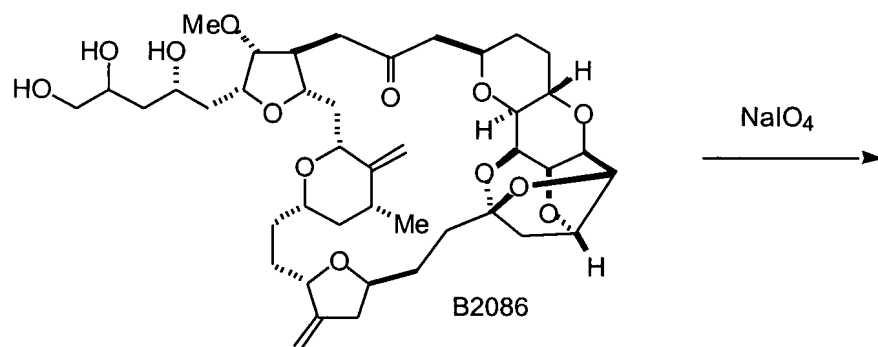
1) MMTrCl  
 2) NaH, MPMCl  
 3) HCl  
 4) PvCl  
 5) AD  
 6) NaIO<sub>4</sub>  
 7) CH<sub>2</sub>=CHCF<sub>2</sub>Br  
 8) In  
 9) OsO<sub>4</sub>  
 10) NaIO<sub>4</sub>  
 11) NaBH<sub>4</sub>  
 12) TBSOTf  
 13) LAH  
 14) Swern  
 15) Wittig  
 16) 9-BBN  
 17) PvCl  
 18) H<sub>2</sub>  
 19) Swern  
 20) Tebbe  
 21) 9-BBN  
 22) Swern  
 23) Et<sub>3</sub>N  
 24) NaBH<sub>4</sub>  
 25) MPMOTCI  
 26) LAH  
 27) separate isomers  
 28) Dess-Martin



Scheme 15



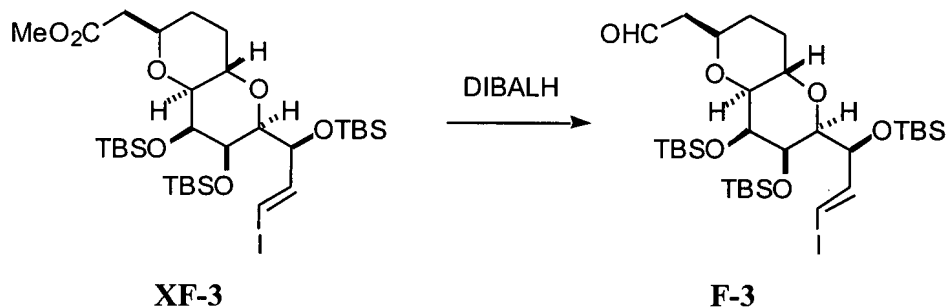
Scheme 16



## EXPERIMENTAL SECTION

### Synthesis of Key Fragment F-3:

5



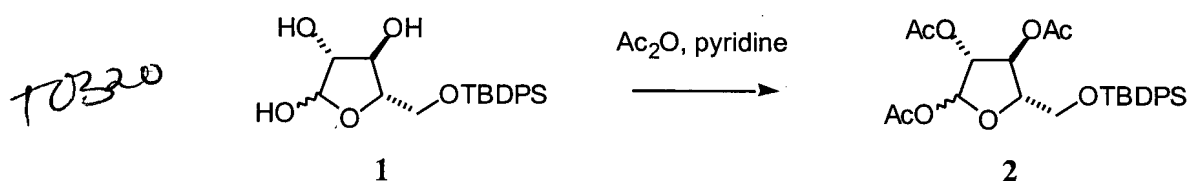
**Key Fragment F-3.** DIBALH (1 M in toluene, 3.86 mL) was added to a solution of **XF-3** (1.46 g, 1.93 mmol) in toluene (37 mL) at -78 °C. After stirring for 10 min, the reaction was quenched by careful addition of MeOH (0.46 mL) and H<sub>2</sub>O (0.21 mL), warmed to rt and stirred for 15 min. The white suspension was filtered through Celite with 1:1 CH<sub>2</sub>Cl<sub>2</sub>/Et<sub>2</sub>O. The filtrate was concentrated and purified by column chromatography (10% EtOAc-hexanes) to give key fragment **F-3** (1.34 g, 96%) as an oil.

15

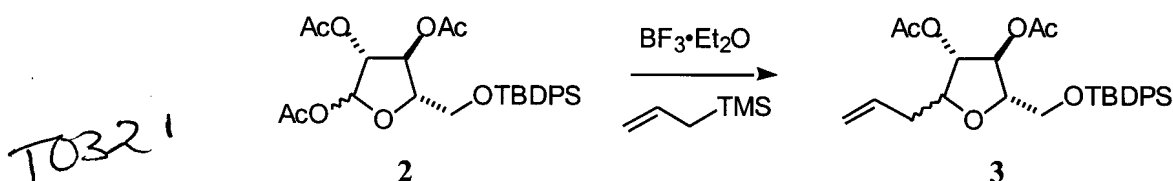
### Synthesis of B1793:



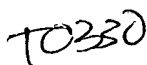
**Triol 1** A solution of TBDPSCl (444 mL, 1.7 mol) in DMF (0.5 L) was added in three portions to a suspension of L-arabinose (250.0 g, 1.66 mol), imidazole (231.4 g, 3.40 mol) and DMF (2.5 L). The addition of each portion took 1.5 h with a 30 min and a 15 h interval separating the second and third portions, respectively. The resulting solution was stirred for 3 h, concentrated and purified by flash chromatography (5% to 33% EtOAc-hexanes) to provide triol **1** (394 g, 61%).



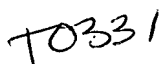
**Triacetate 2** Acetic anhydride (6.06 mol) was added over 1.5 h to triol **1** (1.01 mol) in pyridine (1.0 L) at 15 °C. The solution was stirred for 1 h, concentrated and purified by flash chromatography (15% to 25% EtOAc-hexanes) to afford triacetate **2** (518 g, 97%).



**Diacetates 3** Allyltrimethylsilane (1.11 mol) followed by  $\text{BF}_3 \cdot \text{OEt}_2$  (1.11 mmol) was added over 1.5 h to triacetate **2** (164 g, 0.32 mol) in toluene (1.5 L) at 0 °C. The orange solution was stirred for 1 h at 0 °C and for 2 h at rt. The mixture was slowly poured into saturated aqueous  $\text{NaHCO}_3$  (1.7 L) at 0 °C and stirred for 30 min. The separated aqueous layer was extracted with EtOAc (3 × 600 mL) and the combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by flash chromatography (5% to 10% EtOAc-hexanes) to furnish a mixture of diacetates **3** (108 g, 69%).

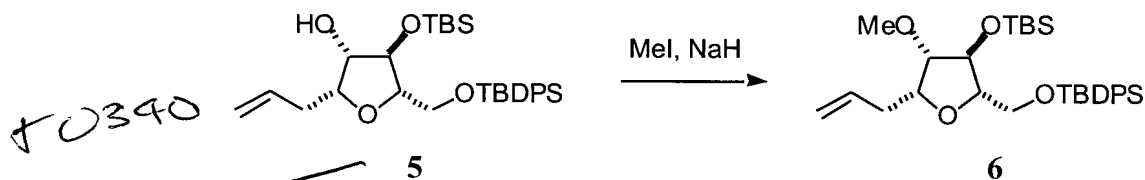


10

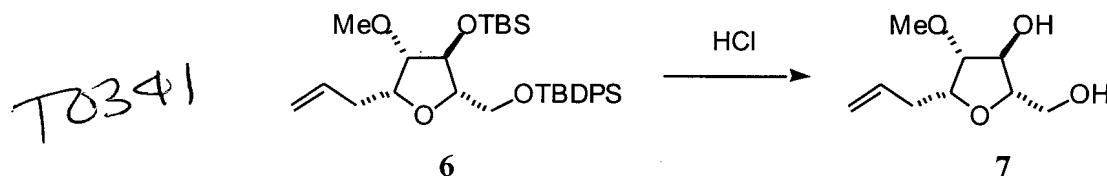


15

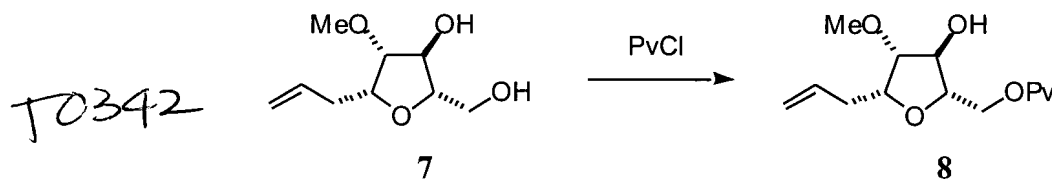




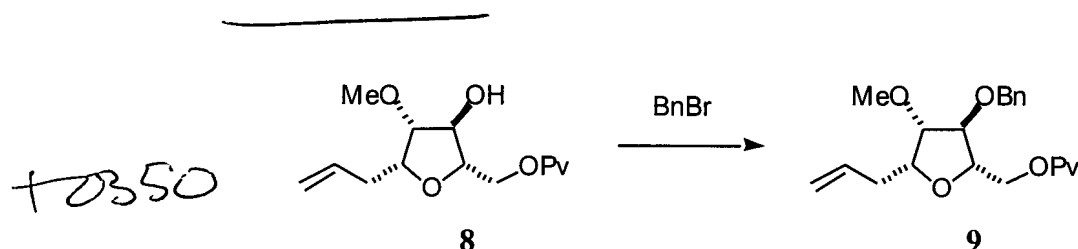
**Methyl ether 6** Iodomethane (16.5 mL, 265 mmol) and NaH (60% in mineral oil, 5.28 g, 132 mmol) were added to a solution of alcohol **5** (34.93 g, 66 mmol), THF (320 mL) and DMF (80 mL) at 0 °C. After 19 h at 0 °C, the reaction was quenched with saturated aqueous  $\text{NH}_4\text{Cl}$  and saturated aqueous  $\text{Na}_2\text{S}_2\text{O}_3$ . The resulting mixture was stirred for 20 min and the layers were allowed to separate. The aqueous phase was extracted with EtOAc (3 × 200 mL) and the combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by flash chromatography (3% EtOAc-hexanes) to afford methyl ether **6** (34.23 g, 96%).



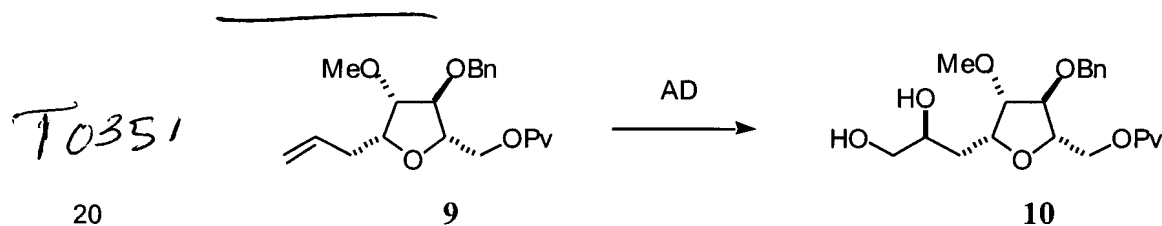
**Diol 7** HCl (37% aqueous solution, 12.75 mL, 153 mmol) was added to a solution of methyl ether **6** (32.93 g, 61 mmol) in MeOH (110 mL) at rt. After 17 h,  $\text{NaHCO}_3$  (17 g) was added to the reaction mixture. The mixture was stirred for 30 min, concentrated, suspended in EtOAc and filtered. The filtrate was concentrated and purified by flash chromatography (50% EtOAc-hexanes to EtOAc) to give diol **7** (10.0 g, 87%).



**Alcohol 8** A solution of pivaloyl chloride (8.4 mL, 67 mmol) in pyridine (50 mL) was added over 1.5 h to a solution of diol 7 (12.24 g, 65 mmol) in pyridine (100 mL) at 0 °C. After 1 h at 0 °C and 18 h at rt, the mixture was diluted with saturated aqueous NH<sub>4</sub>Cl and extracted with EtOAc (3 × 800 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by flash chromatography (50% EtOAc-hexanes) to furnish alcohol 8 (16.9 g, 96%).



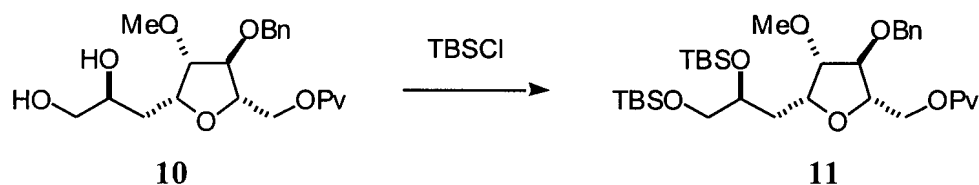
**Olefin 9** Benzyl bromide (62 mL, 521 mmol) and Bu<sub>4</sub>NHSO<sub>4</sub> (10.6 g, 31 mmol) were added to a solution of alcohol 8 (16.9 g, 62 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) at 0 °C. A solution of NaOH (9.95 g, 248 mmol) in H<sub>2</sub>O (10 mL) was added to the reaction mixture over 15 min. After 30 min at 0 °C and 18 h at rt, the reaction mixture was diluted with saturated aqueous NH<sub>4</sub>Cl and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by flash chromatography (hexanes to 30% EtOAc-hexanes) to afford olefin 9 (22.1 g, 98%).



**Diol 10** OsO<sub>4</sub> (0.1 M solution in toluene, 7.3 mL, 0.73 mmol) and a solution of olefin 9 (24.9 g, 69 mmol) in t-BuOH (165 mL) were added to a solution of K<sub>2</sub>CO<sub>3</sub> (31.2 g, 161 mmol), K<sub>3</sub>Fe(CN)<sub>6</sub> (74.4 g, 161 mmol), (DHQ)<sub>2</sub>PYR (1.33 g, 1.50 mmol), H<sub>2</sub>O (500 mL) and t-BuOH (330 mL) at 0 °C. After 3 h at 0 °C, Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> • 5 H<sub>2</sub>O (37.3 g, 150 mmol) was added. The

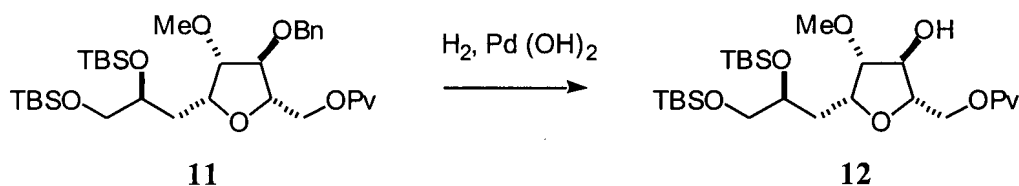
reaction mixture was warmed to rt, stirred for 1 h and extracted with EtOAc (3 × 300 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by flash chromatography (5% isopropanol- CH<sub>2</sub>Cl<sub>2</sub>) to provide diol **10** (17.98 g, 75%).

5

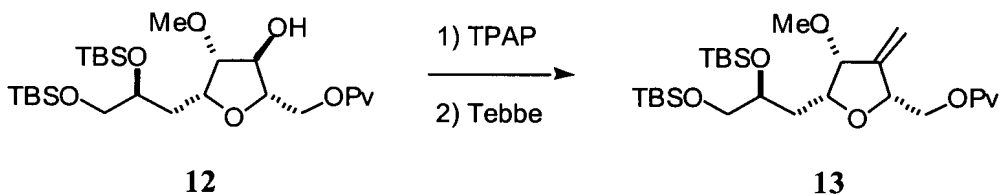


**Silyl ether 11** Imidazole (21 g, 308 mmol) and TBSCl (26.5 g, 176 mmol) were added to a solution of diol **10** (17.4 g, 44 mmol) in DMF (90 mL) at rt. After 18 h, the reaction mixture was diluted with saturated aqueous NaHCO<sub>3</sub> (250 mL), stirred for 1 h and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by flash chromatography (5% EtOAc-hexanes) to afford silyl ether **11** (25.7 g, 94%).

15



**Alcohol 12** A mixture of silyl ether **11** (21.2 g, 33.8 mmol), Pd(OH)<sub>2</sub> (20%, 4.7 g, 33.8 mmol) and EtOAc (200 mL) was stirred at rt under 1 atm H<sub>2</sub> for 3 h. The mixture was filtered through Celite, concentrated and purified by flash chromatography (10% to 20% EtOAc-hexanes) to afford alcohol **12** (17.4 g, 96%).

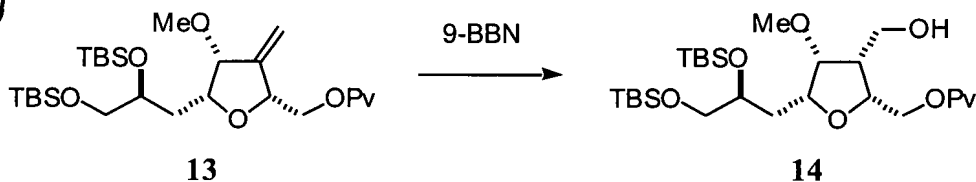


**Olefin 13** 4-Methylmorpholine N-oxide (7.66 g, 65 mmol) and TPAP (1.15 g, 3.26

5 mmol) was added in four portions over 20 min to a solution of alcohol **12** (17.4 g, 32.5 mmol) in  $\text{CH}_2\text{Cl}_2$  (145 mL) at 0 °C. After 20 min, the reaction mixture was diluted with  $\text{Et}_2\text{O}$  (50 mL) and saturated aqueous  $\text{Na}_2\text{S}_2\text{O}_5$  (50 mL) and filtered through Celite. The organic layer was separated, washed sequentially with saturated aqueous  $\text{CuSO}_4$ -brine (1:1) and brine, dried over  $\text{Na}_2\text{SO}_4$ , filtered through Celite, and concentrated to afford the desired crude ketone.

10 Tebbe reagent was prepared by stirring bis(cyclopentadienyl)titanium (11.36 g, 45.6 mmol) and  $\text{Me}_3\text{Al}$  (2.0 M in toluene, 45.6 mL, 91.2 mmol) for 4 days at rt. This material was cooled to -25 °C and a solution of crude ketone in THF (150 mL) was added. The reaction mixture was warmed to 0 °C, stirred for 30 min, quenched by slow addition of 0.1 N NaOH (3.5 mL), and then stirred for an additional 20 min at rt. The mixture was diluted with  $\text{Et}_2\text{O}$ , filtered

15 through Celite and concentrated. The residue was dissolved in  $\text{CH}_2\text{Cl}_2$ , filtered through basic  $\text{Al}_2\text{O}_3$ , concentrated and purified by flash chromatography (5% EtOAc-hexanes) to give olefin **13** (12.8 g, 74% for two steps).

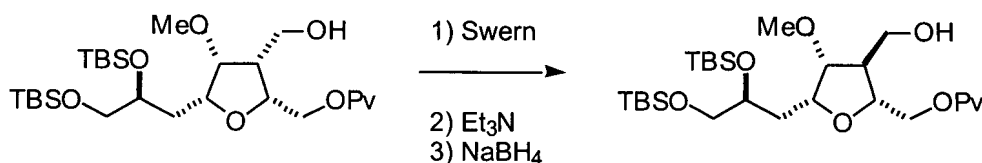


**Alcohol 14** 9-BBN (0.5 M in THF, 165 mL, 83 mmol) was added to a solution of olefin **13** (12.78 g, 24 mmol) in THF (280 mL) at 0 °C. After stirring for 5 h at rt, the reaction mixture

25 was recooled to 0 °C at which time  $\text{H}_2\text{O}$  (200 mL), THF (100 mL) and  $\text{NaBO}_3 \cdot 4 \text{H}_2\text{O}$  (75 g)

were added. The mixture was warmed to rt, stirred for 16 h and then concentrated. The aqueous residue was extracted with EtOAc (4 × 300 mL) and the combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>. Concentration and purification by flash chromatography (20% to 35% EtOAc-hexanes) afforded alcohol **14** (12.05 g, 91%).

5



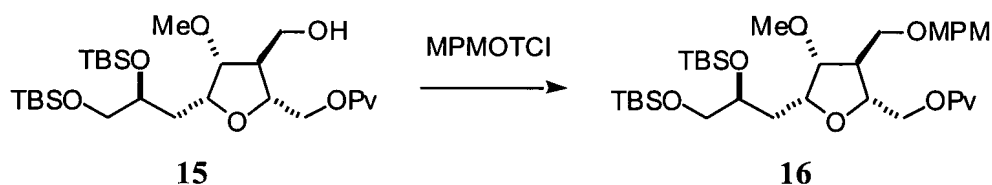
**14**

**15**

**Alcohol 15** DMSO (9 mL, 127 mmol) was added to a solution of oxalyl chloride (5.6 mL, 64 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (350 mL) at -78 °C. After stirring for 15 min, a solution of alcohol **14** (11.7 g, 0.021 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) was added and stirring was continued for 1 h, after which Et<sub>3</sub>N (26.7 mL, 192 mmol) was added. The reaction mixture was warmed to 0 °C, stirred for 15 min, diluted with saturated aqueous NH<sub>4</sub>Cl, and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 200 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to furnish the desired crude aldehyde.

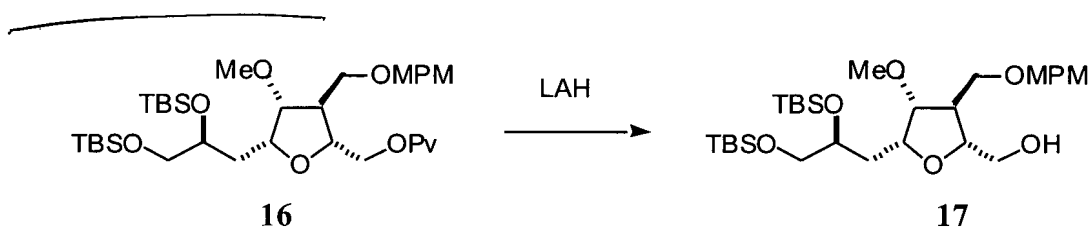
This material was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (200 mL) and treated with Et<sub>3</sub>N (20 mL) at rt. After stirring overnight, the reaction mixture was diluted with saturated aqueous NH<sub>4</sub>Cl and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 200 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and filtered through a short SiO<sub>2</sub> column (20% EtOAc-hexanes) to afford the crude epimerized product.

The aldehyde was dissolved in Et<sub>2</sub>O-EtOH (1:1, 100 mL), cooled to 0 °C and treated with sodium borohydride (1.21 g, 32 mmol). The mixture was stirred for 20 min, carefully diluted with saturated aqueous NH<sub>4</sub>Cl, stirred for 30 min at rt and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 150 mL). The combined extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by flash chromatography (20% EtOAc-hexanes) to afford alcohol **15** (9.95 g, 85% for three steps).



5 **MPM-ether 16**  $\text{BF}_3 \cdot \text{OEt}_2$  (0.1 M in  $\text{CH}_2\text{Cl}_2$ , 1.8 mL, 0.18 mmol) was added to a solution of alcohol **15** (9.87 g, 18 mmol), MPM-trichloroimidate (4.9 mL, 27 mmol) and  $\text{CH}_2\text{Cl}_2$  (175 mL) at 0 °C. After 40 min, a second portion of  $\text{BF}_3 \cdot \text{OEt}_2$  (0.1 M in  $\text{CH}_2\text{Cl}_2$ , 0.9 mL, 0.09 mmol) was added to the reaction mixture. After 20 min, the reaction was quenched with saturated aqueous  $\text{NH}_4\text{Cl}$ , stirred for 1 h at rt and diluted with  $\text{Et}_2\text{O}$  (600 mL). The organic layer

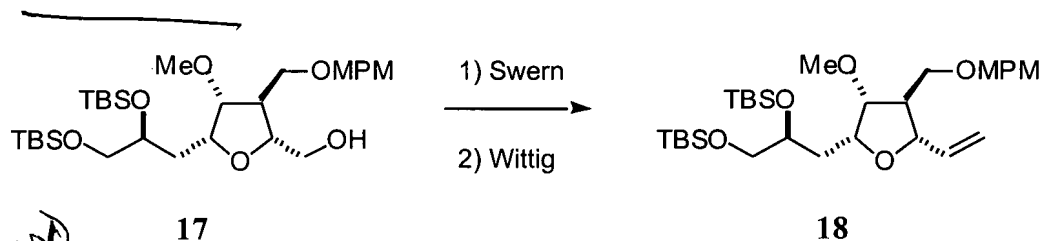
10 was separated and the aqueous layer was extracted with  $\text{Et}_2\text{O}$  (150 mL). The combined organic extracts were washed sequentially with 0.1 N aqueous  $\text{NaOH}$ , saturated aqueous  $\text{NaHCO}_3$ , brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by flash chromatography (20%  $\text{EtOAc}$ -hexanes) to give MPM-ether **16** (10.20 g, 85%).



70400

**Alcohol 17** LAH (1 M in THF, 22.5 mL, 22.5 mmol) was added to a solution of MPM-  
 5 ether **16** (10.05 g, 15 mmol) in Et<sub>2</sub>O (1.0 L) at 0 °C. After 30 min, the reaction was cautiously  
 quenched with H<sub>2</sub>O (1.3 mL), and 1 N aqueous NaOH (1.3 mL). After stirring for 1 h at rt, the  
 suspension was filtered through Celite, concentrated and purified by flash chromatography (20%  
 EtOAc-hexanes) to afford alcohol **17** (8.18 g, 93%).

10

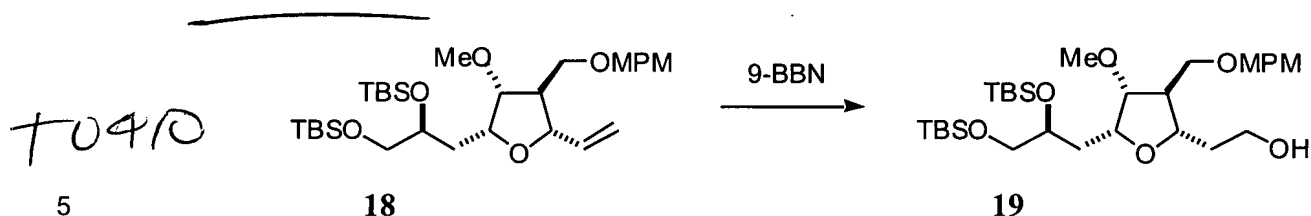


70400

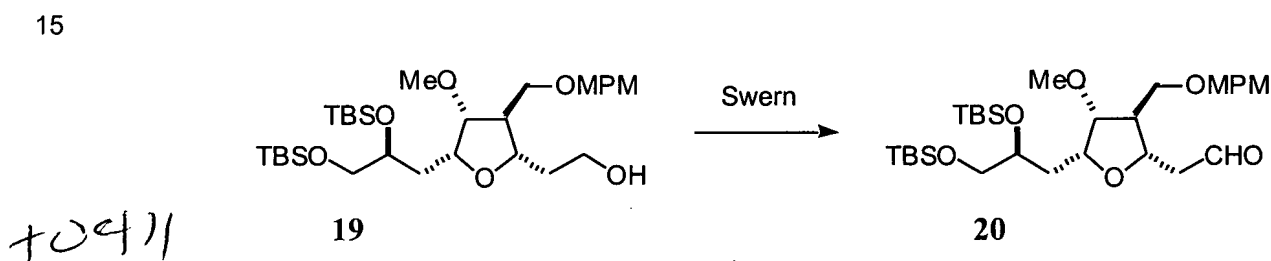
**Olefin 18** DMSO (5.8 mL, 82.4 mmol) was added to a solution of oxalyl chloride (3.6  
 15 mL, 41.2 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) at -78 °C. After 15 min, a solution of alcohol **17** (7.94 g,  
 13.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (35 mL) was added to the reaction mixture. After stirring for 1 h, Et<sub>3</sub>N (17  
 mL, 122 mmol) was added, the mixture was warmed to 0 °C, stirred for 20 min, diluted with  
 saturated aqueous NH<sub>4</sub>Cl and then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 100 mL). The combined organic  
 extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and filtered through a short SiO<sub>2</sub> column (20%  
 20 EtOAc-hexanes) to furnish the desired crude aldehyde.

*n*-BuLi (1.6 M, 20 mL, 30 mmol) was added dropwise to a solution of CH<sub>3</sub>PPh<sub>3</sub>Br (10.1  
 g, 30 mmol) in THF (350 mL) and DMSO (100 mL) at 0 °C. After 1 h, a solution of the crude  
 aldehyde in THF (50 mL) was added. The reaction mixture was warmed to rt and stirred for 3 h.  
 Saturated aqueous NH<sub>4</sub>Cl was added and the mixture was extracted with EtOAc (3 × 500 mL).

The combined extracts were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by flash chromatography (7% EtOAc-hexanes) to afford olefin **18** (5.57 g, 71% yield for 2 steps).



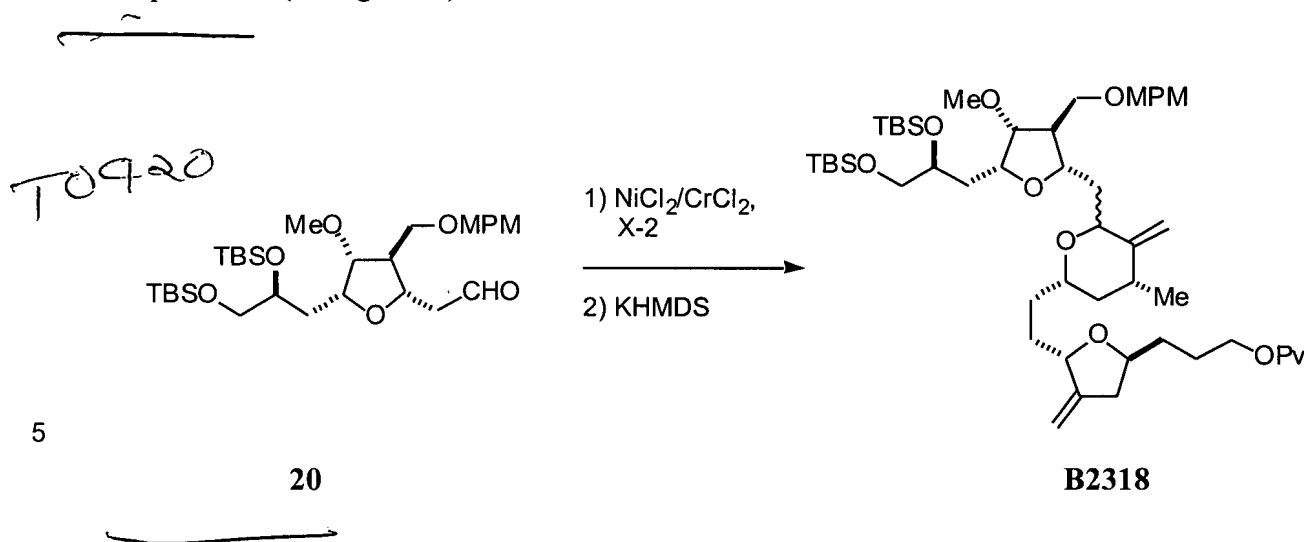
**Alcohol 19** 9-BBN (0.5 M in THF, 65 mL, 33 mmol) was added to a solution of olefin **18** (5.56 g, 9.6 mmol) in THF (85 mL) at 0 °C. The mixture was stirred for 5 h at rt and then recooled to 0 °C.  $\text{H}_2\text{O}$  (200 mL), THF (100 mL), and  $\text{NaBO}_3 \cdot 4 \text{H}_2\text{O}$  (30 g) were sequentially added. After stirring overnight at rt, the organic volatiles were removed under reduced pressure. The aqueous residue was extracted with EtOAc (3 × 200 mL) and the combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ . Concentration and purification by flash chromatography (30% EtOAc-hexanes) afforded alcohol **19** (12.05 g, 92%).



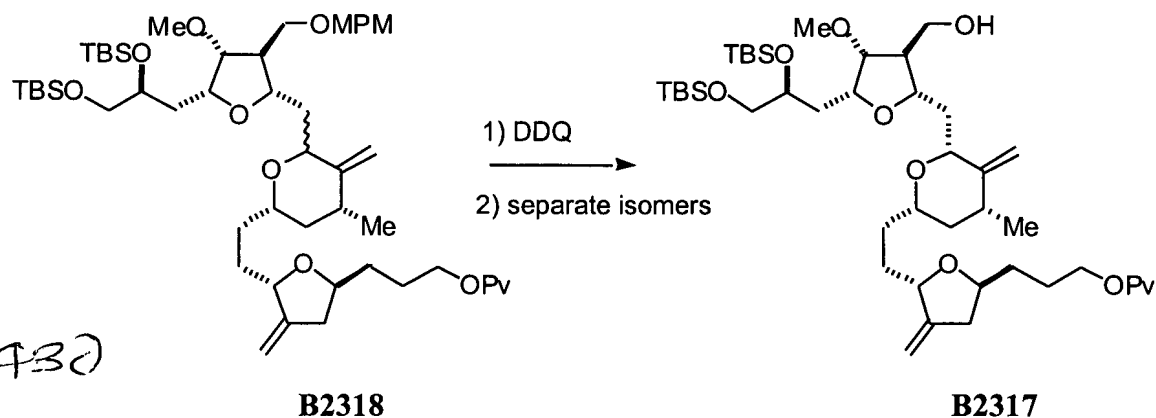
**Aldehyde 20** DMSO (1.36 mL, 19.2 mmol) was added dropwise over 4 min to a solution of oxalyl chloride (1.26 mL, 14.4 mmol) in  $\text{CH}_2\text{Cl}_2$  (120 mL) at -78 °C. After stirring for 10 min, a solution of alcohol **19** (5.76 g, 9.61 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL) was added via cannula. The transfer was completed by rinsing with additional  $\text{CH}_2\text{Cl}_2$  (2 × 5 mL). After stirring for 20 min, the mixture was treated with  $\text{Et}_3\text{N}$  (5.36 mL, 38.4 mmol) and stirred for 10 min at -78 °C, 30 min at 0 °C and 10 min at rt. The reaction mixture was poured into saturated aqueous  $\text{NaHCO}_3$  (200 mL) and the separated aqueous layer was extracted with  $\text{CH}_2\text{Cl}_2$  (3×) followed by



EtOAc (100 mL). The combined organic phases were dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by column chromatography (10% to 20% EtOAc-hexanes) to furnish aldehyde compound **20** (5.28 g, 92%) as an oil.



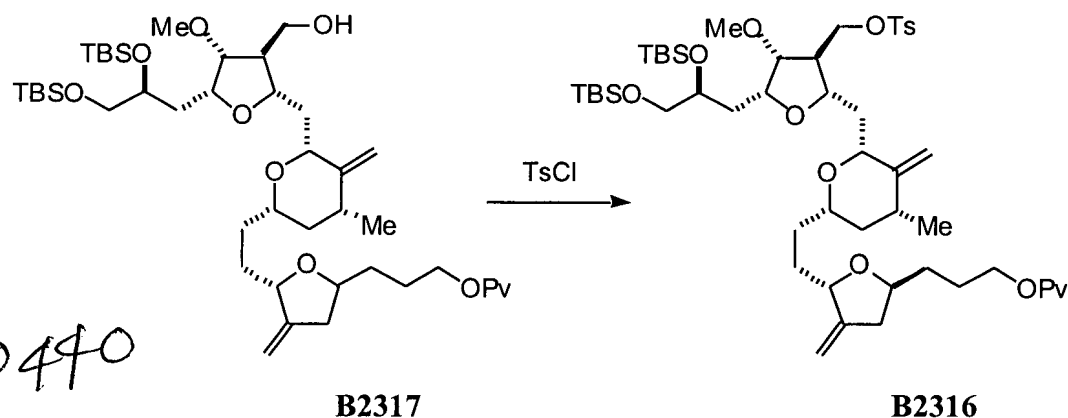
**B2318** 0.1%  $\text{NiCl}_2/\text{CrCl}_2$  (w/w, 3.21 g) and 1%  $\text{NiCl}_2/\text{CrCl}_2$  (w/w, 4.31 g) was added to a solution of aldehyde **20** (3.73 g, 6.25 mmol), key fragment **F-2** exemplified by vinyl iodide **X2** (5.10 g, 9.16 mmol), THF (85 mL) and DMF (21 mL) at rt in a glove box. The reaction mixture was stirred for 24 h, removed from the glove box, cooled to 0 °C, diluted with EtOAc (100 mL), quenched with saturated  $\text{NH}_4\text{Cl}$  (200 mL) and stirred for 30 min. The separated aqueous phase was extracted with EtOAc (6×) and the combined organic layers were dried over  $\text{Na}_2\text{SO}_4$  concentrated and purified by column chromatography (20% to 30%) to give **B2318** (~3 g) contaminated with close running impurities and the uncyclized intermediate (4.61 g). The latter (4.61 g, 4.48 mmol,) was dissolved in THF (150 mL), cooled to 0 °C and treated with KHMDS (0.5 M in toluene, 14 mL, 7.0 mmol) over a 2 min period. After stirring at 0 °C for 15 min, the reaction was quenched with saturated aqueous  $\text{NH}_4\text{Cl}$  (150 mL) and warmed to rt. The separated aqueous layer was extracted with EtOAc (3×) and the combined organic phases were dried over  $\text{Na}_2\text{SO}_4$ , concentrated and combined with the partially purified product obtained above. Column chromatography (10% EtOAc-hexanes) afforded **B2318** (3.17 g, 55%) as an inseparable ~3:1 mixture of C27 diastereomers.



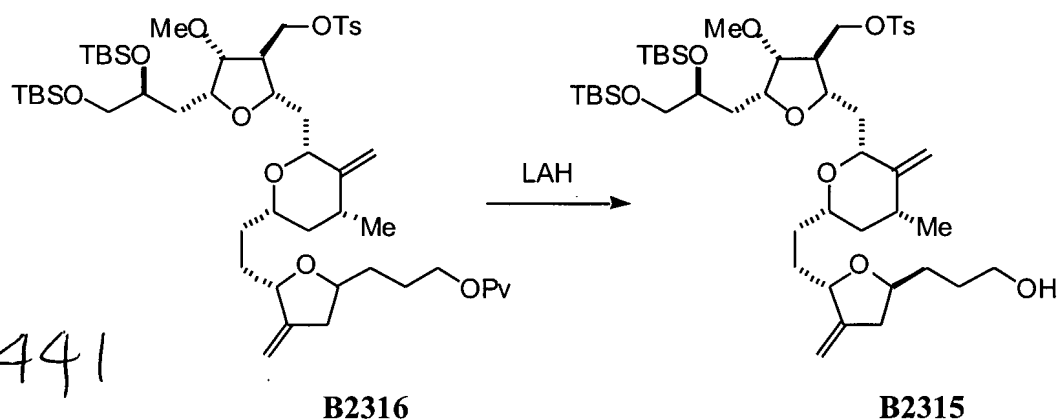
5 **B2317** DDQ (1.45 g, 6.42 mmol) was added portion-wise over 30 min to a stirred solution of **B2318** (3.12 g, 3.35 mmol) in  $\text{CH}_2\text{Cl}_2$  (50 mL) and pH 7 phosphate buffer (5 mL) at rt. The reaction was quenched with saturated aqueous  $\text{NaHCO}_3$  (50 mL), stirred for 5 min, diluted with additional saturated aqueous  $\text{NaHCO}_3$  (100 mL),  $\text{H}_2\text{O}$  (200 mL) and extracted with  $\text{Et}_2\text{O}$  (5 $\times$ ). The combined organic phases were dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by

10 column chromatography (15% to 30% EtOAc-hexanes) to give recovered **B2318** (1.40 g) and a mixture of the C27 isomeric products. The recovered **B2318** was resubmitted to the reaction conditions described above to afford additional product. Recovered starting material was again cycled through the deprotection conditions. All of the desired material was combined and separated by MPLC to afford **B2317** (1.65 g, 61%).

15



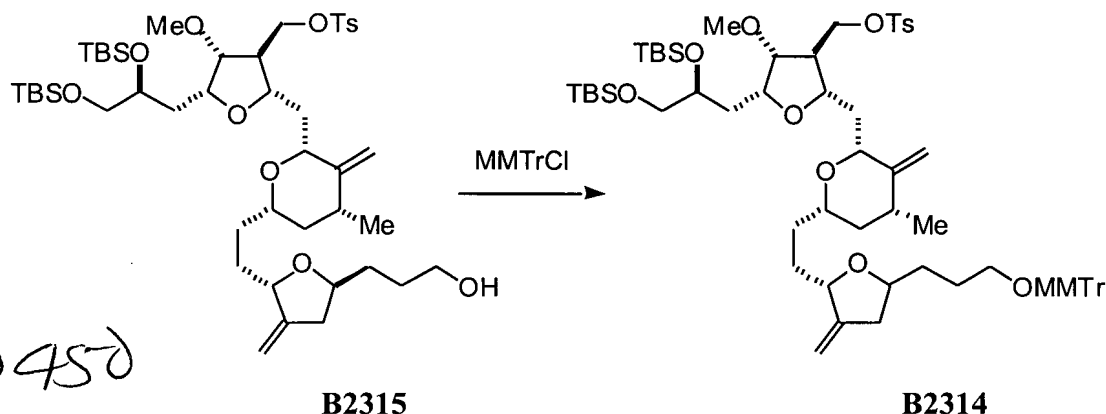
- 5 **B2316** TsCl (0.63 g, 3.30 mmol) was added to a solution of **B2317** (1.60 g, 1.97 mmol) in  $\text{CH}_2\text{Cl}_2$  (8 mL) and pyridine (2 mL) at rt. After stirring for 29 h, the reaction was quenched with saturated aqueous  $\text{NaHCO}_3$  (30 mL) and  $\text{H}_2\text{O}$  (10 mL). The separated aqueous layer was extracted with  $\text{Et}_2\text{O}$  and the combined organic layers were dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by column chromatography (15% to 30% EtOAc-hexanes) to give **B2316** (2.01 g, 92%)
- 10 as an oil along with recovered **B2317** (92 mg, 5.8%).



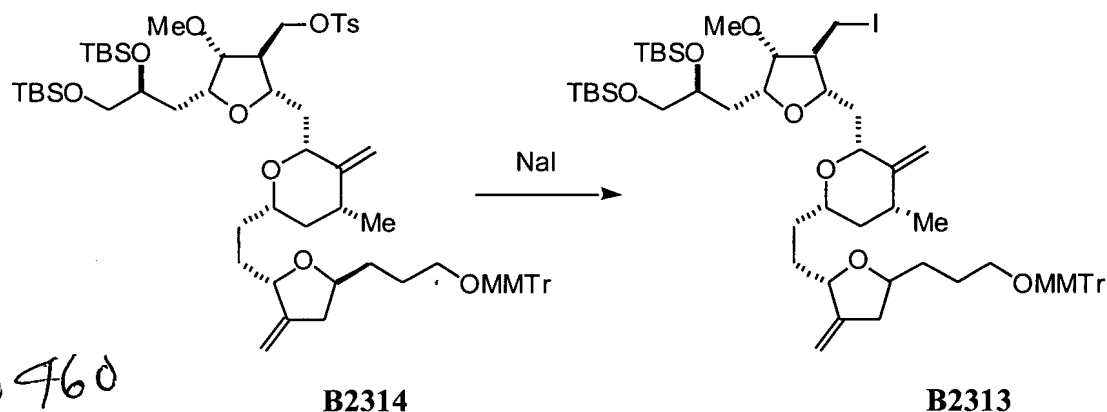
- 15 **B2315** LAH (1 M in THF, 2.61 mL, 2.61 mmol) was added over 1 min to a solution of **B2316** (1.68 g, 1.74 mmol) in  $\text{Et}_2\text{O}$  (80 mL) at  $0^\circ\text{C}$ . After stirring for 7 min, the reaction was

quenched by careful addition of MeOH (0.42 mL, 10.4 mmol) and H<sub>2</sub>O (0.19 mL, 10 mmol), warmed to rt and stirred for 20 min. Filtration through Celite with 1:1 CH<sub>2</sub>Cl<sub>2</sub>-Et<sub>2</sub>O, concentration and purification by column chromatography (30% to 40% EtOAc-hexanes) gave **B2315** (1.38 g, 90%) as an oil.

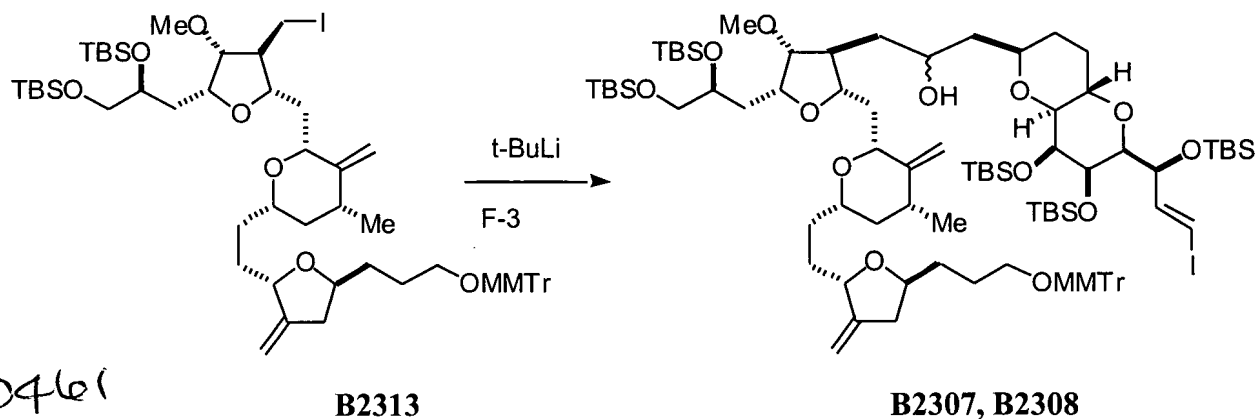
5



- 10 **B2314** MMTrCl (0.70 g, 2.26 mmol) was added to a solution of **B2315** (1.33 g, 1.51 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (25 mL) and iPr<sub>2</sub>NEt (0.79 mL, 4.53 mmol) at rt. The resulting mixture was stirred for 1 h and then poured into a mixture of saturated aqueous NaHCO<sub>3</sub> (20 mL), H<sub>2</sub>O (10 mL) and Et<sub>2</sub>O (50 mL). The separated aqueous layer was extracted with Et<sub>2</sub>O (3×). The combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column
- 15 chromatography (CH<sub>2</sub>Cl<sub>2</sub> followed by 15% to 30% EtOAc-hexanes) to give **B2314** (1.65 g, 95%) as a solid foam.



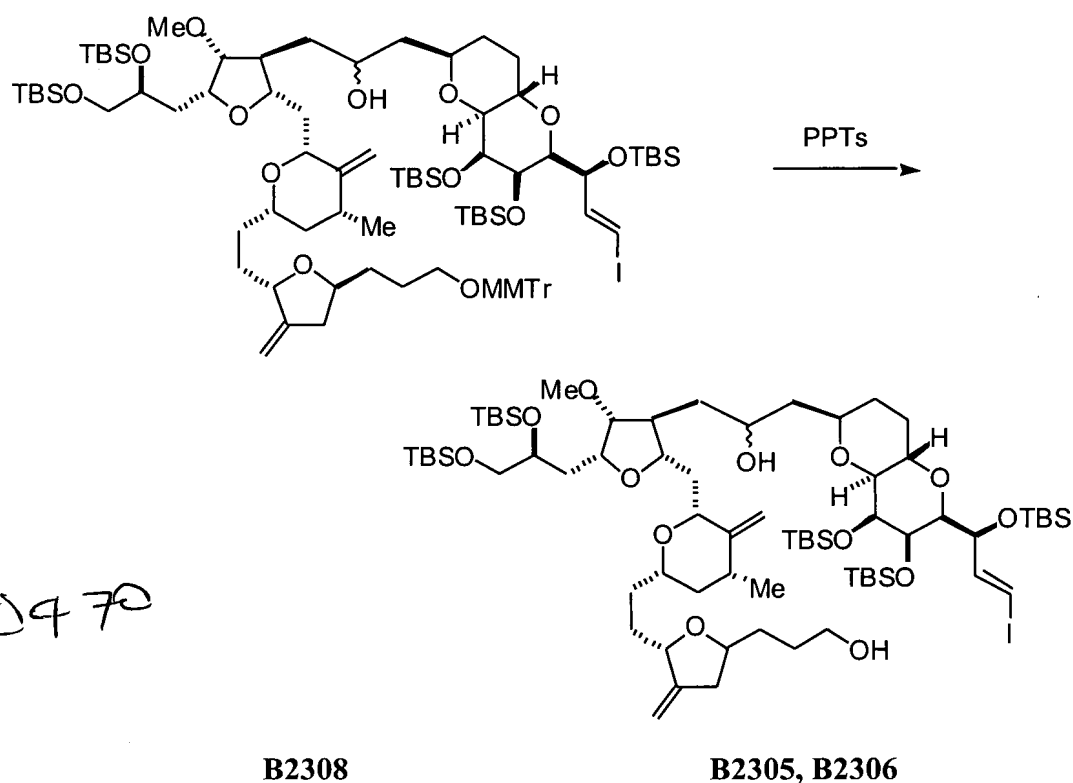
**B2313** A mixture of **B2314** (1.60 g, 1.39 mmol) and NaI (3.10 g, 20.8 mmol) in acetone (50 mL) was heated under reflux for 13 h. After cooling to rt, the reaction mixture was diluted with EtOAc, and concentrated. H<sub>2</sub>O (5 mL), brine (20 mL) and Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (200 mg) were added and the resulting mixture was extracted with Et<sub>2</sub>O (4×). The combined extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (10% EtOAc-hexanes) to give **B2313** (1.50 g, 97%) as an oil.



**B2308** *Tert*-BuLi (1.7 M in pentane, 1.00 mL, 1.7 mmol) was added over 1 min to a solution of **B2313** (0.90 g, 0.81 mmol) in Et<sub>2</sub>O (14 mL) at -78 °C. After stirring for 9 min, the mixture was transferred via cannula over 4 min to a solution of key fragment F-3 (0.83 g, 1.14

mmol) in Et<sub>2</sub>O (4 mL) at -78 °C. The transfer was completed by rinsing with additional Et<sub>2</sub>O (2 mL). The resultant mixture was stirred at -78 °C for 5 min and then at 0 °C for 10 min, quenched with saturated aqueous NaHCO<sub>3</sub> (30 mL) and warmed to rt. The separated aqueous layer was extracted with Et<sub>2</sub>O (3×) and the combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The residue was combined with those of other two batches (corresponding to 0.11 g and 0.44 g of **B2313**) and purified by column chromatography (10% to 20% EtOAc-hexanes) to give a mixture of **B2307** and **B2308** (1.86 g, 83%) as a solid foam. Although the isomers could be separated by prep TLC (20% EtOAc-hexanes), they were carried forward as a mixture.

10

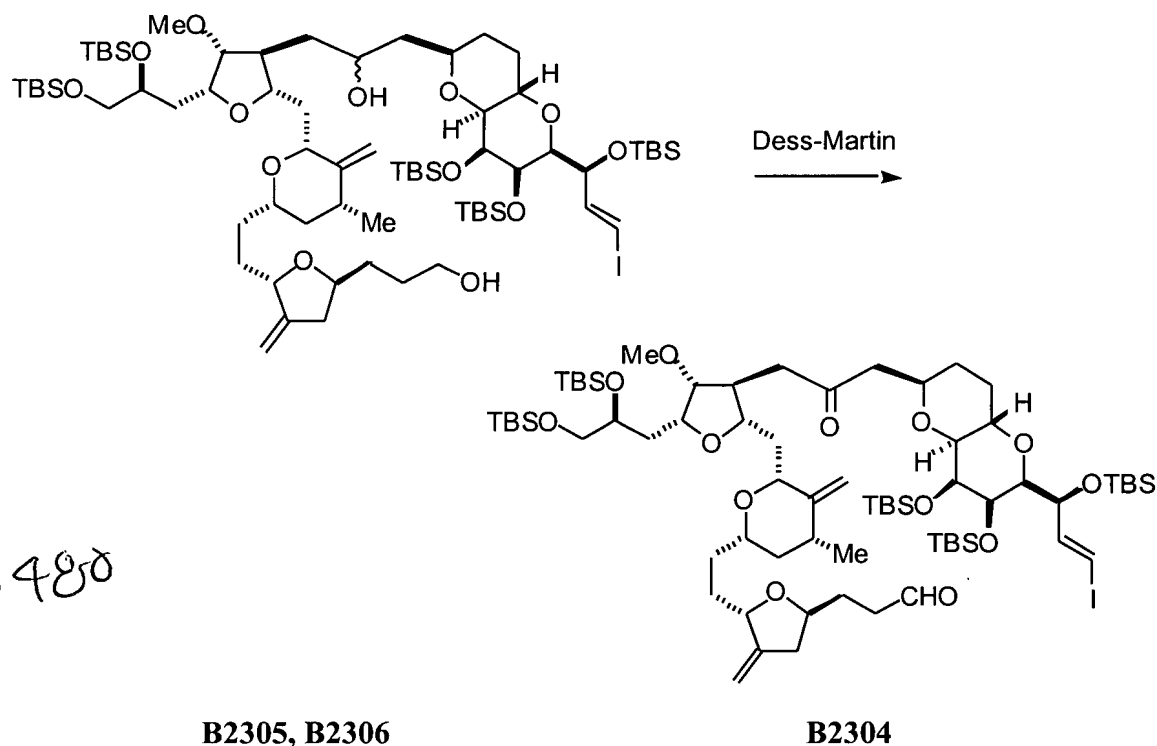


T0470

**B2305 and B2306** The mixture of **B2307/B2308** (1.80 g, 1.05 mmol) was dissolved in EtOH (20 mL), treated with PPTS (10.0 mg, 0.04 mmol), stirred at rt for 11 h and then quenched with NaHCO<sub>3</sub> (20.0 mg, 0.24 mmol). After stirring for 15 min, the mixture was concentrated,

azeotroped with toluene (15 mL), and purified by column chromatography (20% to 30% EtOAc-hexanes) to give a mixture of **B2305** and **B2306** (1.22 g, 81%) as a solid foam. Although the isomers could be separated by prep TLC (30% EtOAc-hexanes), they were carried forward as a mixture.

5

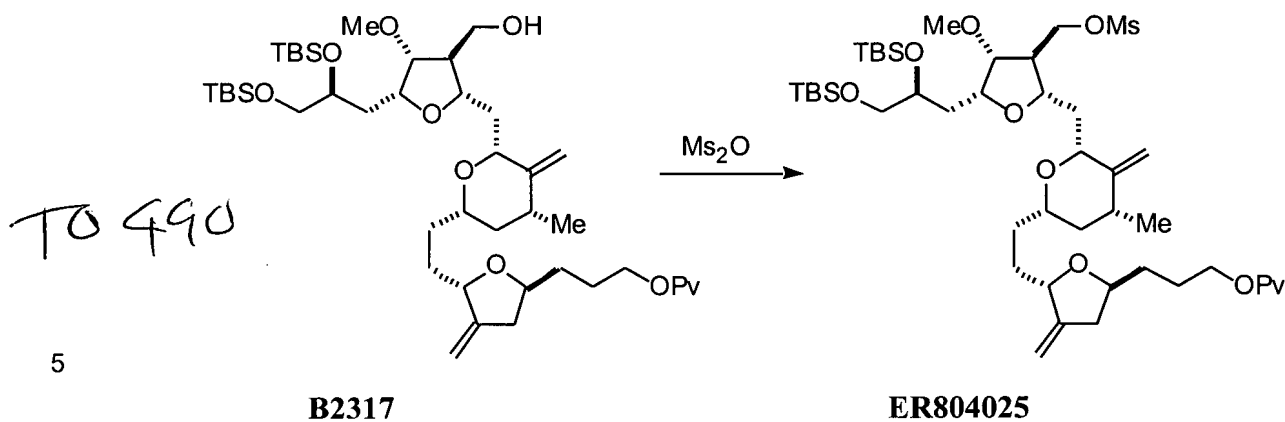


TG 480

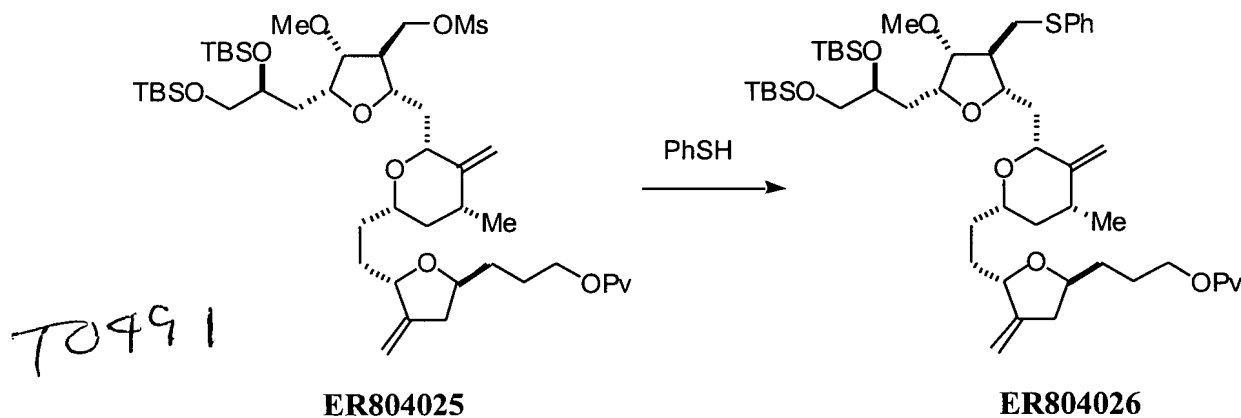
10 **B2304** A mixture of **B2305/B2306** (1.16 g, 0.68 mmol), and Dess-Martin periodinane (0.61 g, 1.44 mmol) in  $\text{CH}_2\text{Cl}_2$  (35 mL) was stirred at rt for 1 h. Additional Dess-Martin periodinane (0.54 g, 1.27 mmol) was added to the mixture and stirring was continued for an additional 1 h. The mixture was diluted with  $\text{Et}_2\text{O}$  (100 mL), stirred for 20 min and filtered through Celite with  $\text{Et}_2\text{O}$ . The colorless filtrate was washed with saturated aqueous  $\text{NaHCO}_3$

15 (100 mL) and the separated aqueous layer was extracted with  $\text{Et}_2\text{O}$  (3X). The combined organic phases were dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by column chromatography (10% to 15% EtOAc-hexanes) to give **B2304** (0.98 g, 84%) as a solid foam.

Alternatively, **B2304** may be prepared as follows and in fact the synthesis described below is superior to that given above.



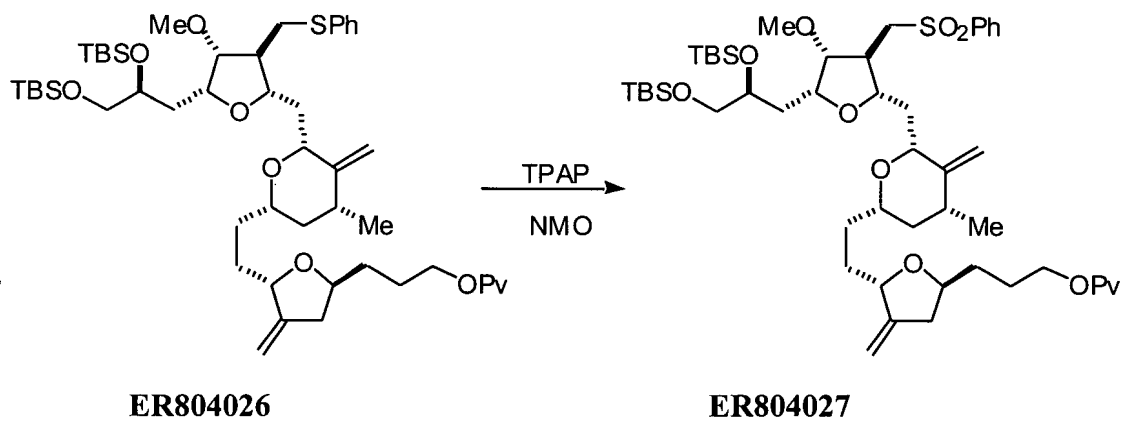
To a solution of the alcohol, 2.4 g mg, in methylene chloride, 29 mL, was added triflic anhydride, 770 mg. The mixture was stirred for 15 minutes, extracted with saturated sodium bicarbonate, dried and chromatographed to give 2.737 g, 100%.



To a solution of the mesylate, 405 mg, in DMF, 0.06 mL, was added di-  
 15 isopropylethylamine, 0.130 mL, followed by benzenethiol, 0.061 mL. After 4 hours and after 22 hours, additional amine, 0.03 mL, and benzenethiol, 0.015 mL, were added. After 24 hours, the mixture was diluted with 5% ethyl acetate/hexane, 1 mL and chromatographed to give 409 mg.



T0500

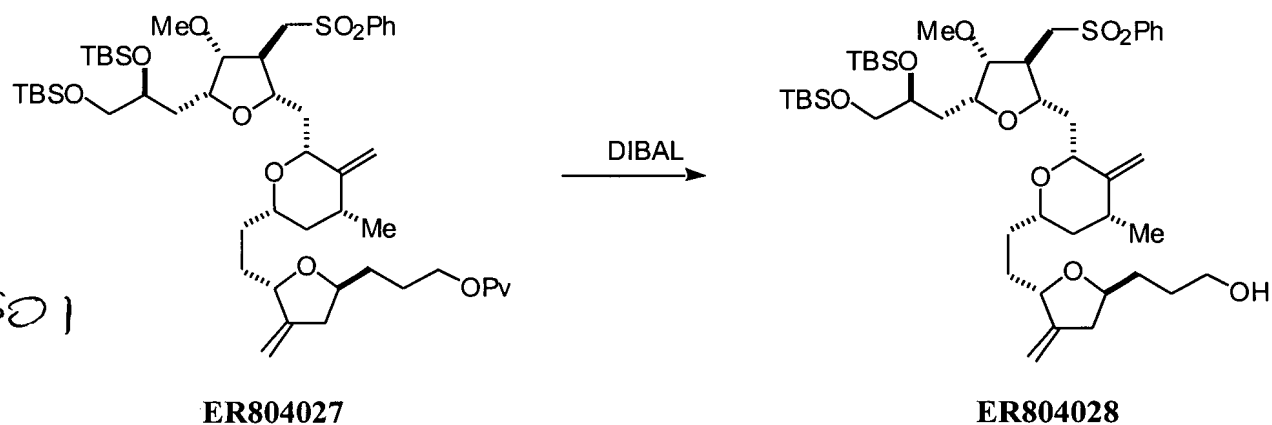


5

To a solution of the sulfide, 1.97 g, in acetonitrile, 16 mL, was added N-methylmorpholine oxide (NMO), and then a solution of 1.02 g, tetrapropylammonium perruthenate(VII), (TPAP), 38 mg, in acetonitrile, 1 mL. After 3.5 hours at room temperature, the mixture was heated to 40 °C for 1 hour. The mixture was cooled and aqueous satd. Sodium

10 thiosulfate was added and the mixture partitioned between water and ethyl acetate. The usual work-up gave 1.567 g of a brown oil.

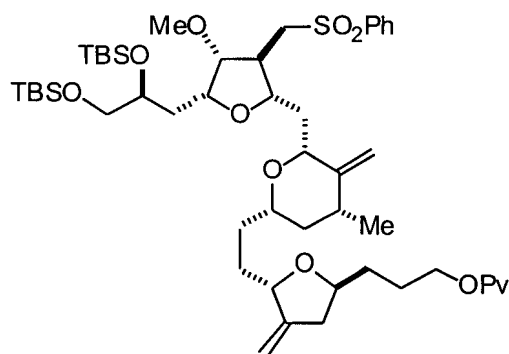
T0501



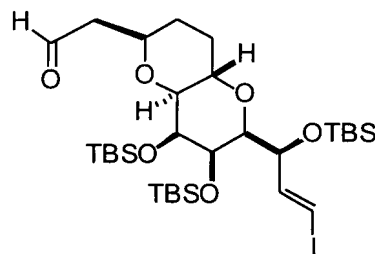
15

To a solution of the pivalate ester, 1.567 g, in methylene chloride, 11.2 mL, at - 78 °C in was added DIBAL, 2.5 mL of a 1 M solution in toluene. After 15 minutes, additional DIBAL,

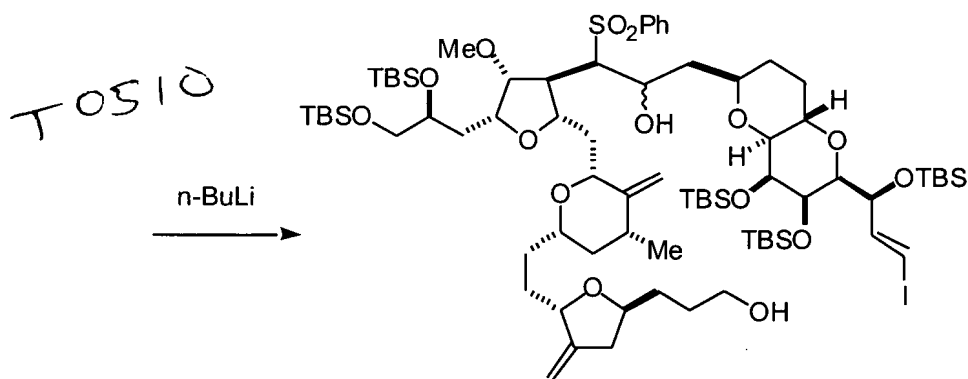
0.8 mL, was added. After an additional 5 minutes, methanol, 0.46 mL, was slowly added followed by water, 0.2 mL. The mixture was filtered through Celite and chromatographed to give 1.386 g of an oil.



ER804028

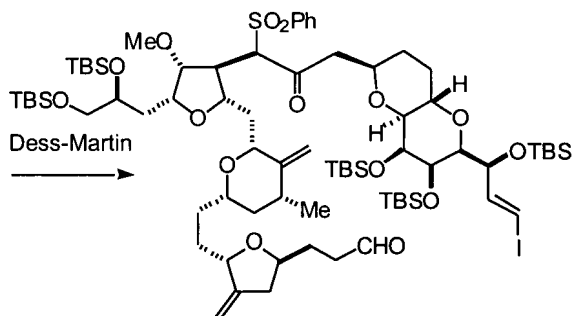
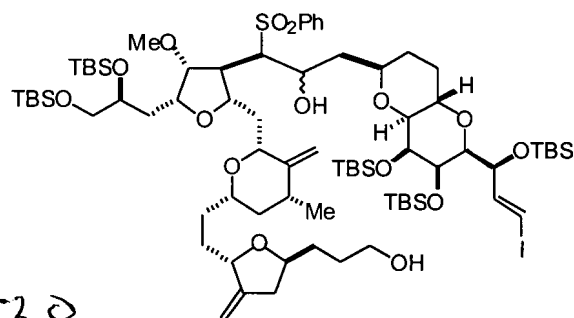


F-3

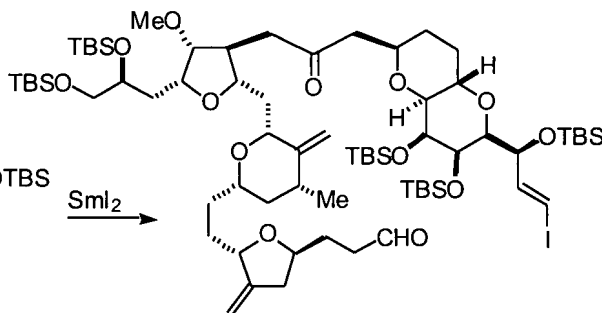
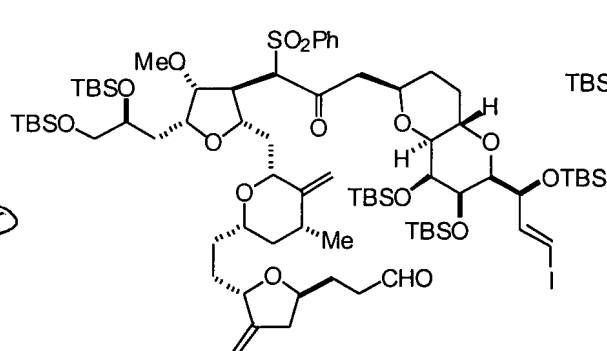


ER804029

To a solution of the sulfone, 36 mg, in DME, 1 mL, at -40 ° C was added n-butyllithium, 2.8 equivalents. After 35 minutes, a solution of the aldehyde, 42 mg, in DME, 0.5 mL) was added. After 40 minutes, saturated aqueous ammonium chloride was added and the mixture extracted with ethyl acetate. The usual work-up, followed by chromatography gave 52 mg of an oil.



To a solution of the alcohol, 42 mg, in methylene chloride, 2 mL, was added the Dess  
 5 Martin reagent, 36.4 mg. The mixture was stirred for 30 minutes and ether was added. The  
 mixture was filtered through Celite, washed with saturated sodium bicarbonate, with saturated  
 sodium thiosulfate, worked up in the usual way and chromatographed to give 38 mg of an oil.



10

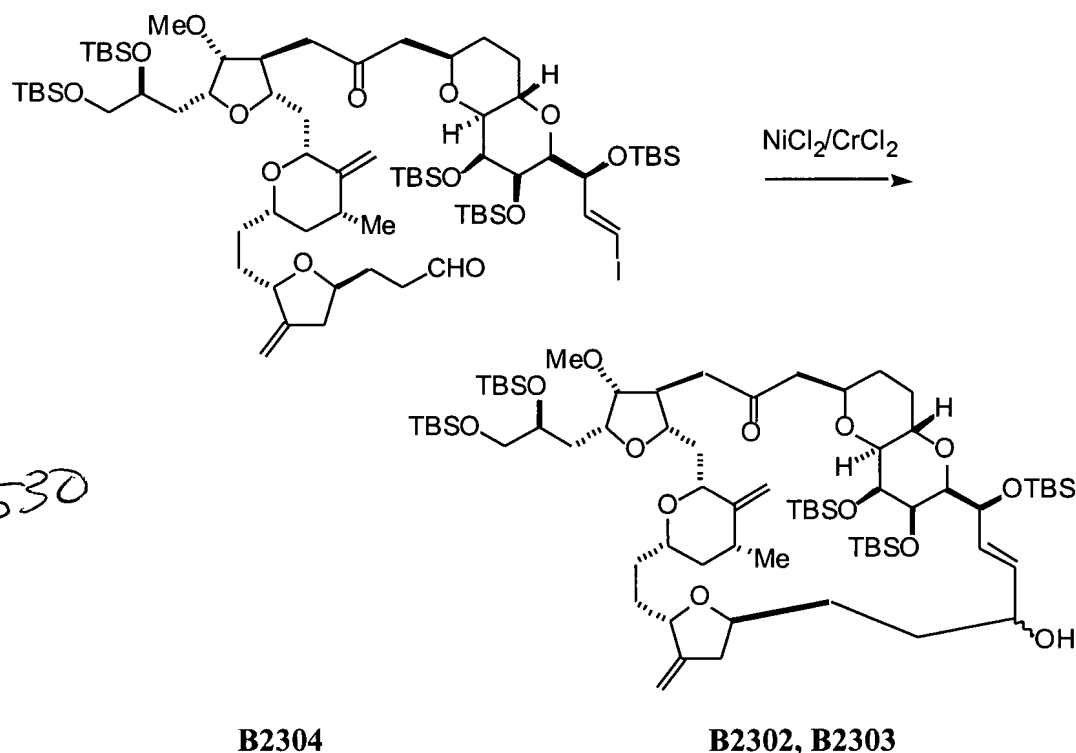
#### Preparation of SmI<sub>2</sub> Solution

A solution of 1,2-di-iodoethane in 10 mL of THF was added to a suspension of Sm, 0.16  
 g, in THF, 1 mL. The mixture was stirred for 1 hour.

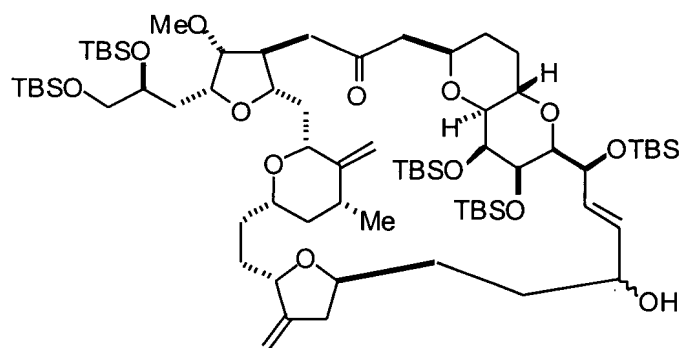
15

An aliquot of this solution, 0.03 mL, was added to a solution of the sulfone in THF at  
 -78 ° C. After 5 minutes, additional SmI reagent, 0.05 mL, as added. After a few additional

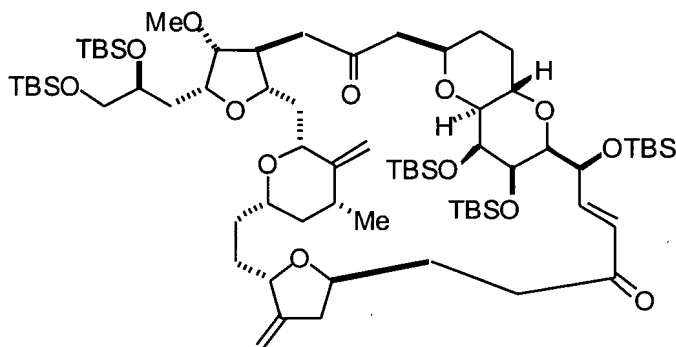
minutes, more reagent, 0.25 mL, was added. The cooling bath was removed and saturated aqueous sodium bicarbonate, 3 mL, was added. The mixture was partitioned between ether and water and the usual work-up gave 9.1 mg, 81%, of an oil.



**B2302 and B2303** In a glove box,  $\text{NiCl}_2/\text{CrCl}_2$  (1% w/w, 1.09 g, 8.86 mmol) was added to a solution of **B2304** (1.01 g, 0.70 mmol) in THF (600 mL) and DMF (150 mL) at rt. After stirring for 2 days the reaction mixture was taken out of the glove box, cooled to 0 °C, quenched with saturated aqueous  $\text{NH}_4\text{Cl}$  (300 mL) and stirred at 0 °C for 20 min. After addition of  $\text{H}_2\text{O}$  (100 mL), the two layers were separated and the aqueous layer was extracted with EtOAc (5×). The combined organic phases were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by column chromatography (15% EtOAc-hexanes) to furnish a mixture of **B2302** and **B2303** (0.84 g, 92%) as a solid foam. Although the isomers could be separated by prep TLC (20% EtOAc-hexanes), they were carried forward as a mixture.



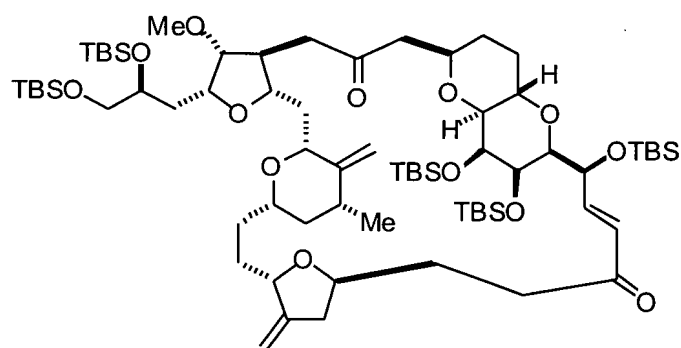
Dess-Martin



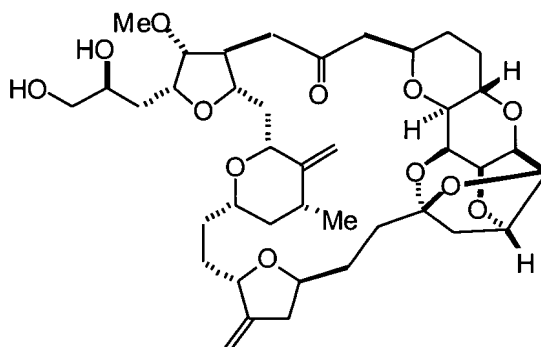
**B2302, B2303**

**B2301**

**B2301** A mixture of **B2302/B2303** (0.79 g, 0.60 mmol) and Dess-Martin periodinane (0.26 g, 0.60 mmol) in  $\text{CH}_2\text{Cl}_2$  (30 mL) at rt was stirred for 30 min. Additional Dess-Martin periodinane (0.26 g, 0.60 mmol) was added to the mixture and stirring was continued for additional 1.5 h. The mixture was then diluted with  $\text{Et}_2\text{O}$  (100 mL), stirred for 15 min and filtered through Celite. The filtrate was washed with saturated aqueous  $\text{NaHCO}_3$  (100 mL) and the separated aqueous layer was extracted with  $\text{Et}_2\text{O}$  (3 $\times$ ). The combined organic phases were dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by column chromatography (10% to 15%  $\text{EtOAc}$ -hexanes) to give **B2301** (0.67 g, 85%) as an oil.



1) TBAF  
2) PPTS



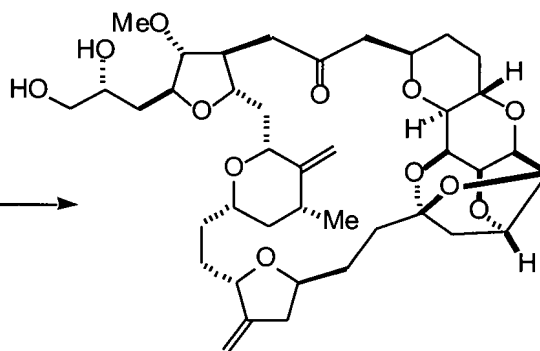
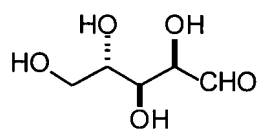
**B2301**

**B1793**

TU550

**B1793** TBAF (1 M in THF containing 0.5 M imidazole HCl, 4.60 mL, 4.60 mmol) was added over 2 min to a solution of **B2301** (0.62 g, 0.48 mmol,) in THF (29 mL) at rt and the resulting mixture was stirred for 18 h. After dilution with hexanes (10 mL), the reaction mixture was directly loaded onto a SiO<sub>2</sub> column packed with 50% EtOAc-hexanes and eluted with 50% EtOAc-hexanes (1 L) followed by 10% MeOH/EtOAc to collect a mixture of intermediates. After solvent removal, the residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) and treated with PPTS (645 mg). After stirring for 1 h at rt, additional PPTS (414 mg) was added and the resulting white suspension was stirred for 4.5 h. The reaction mixture was then directly loaded onto a SiO<sub>2</sub> column packed with 70% EtOAc-hexanes and eluted with 70% EtOAc/hexanes (0.5 L), EtOAc (1 L). Elution with 5% to 10% MeOH/EtOAc furnished pure **B1793** (181 mg) and elution with 15% MeOH-EtOAc gave additional semi-pure product, which after purification by preparative TLC (10% MeOH-EtOAc) provided additional pure **B1793** (42 mg). **B1793** (total 223 mg, 64%) was obtained as a white solid. HRMS: calcd for C<sub>40</sub>H<sub>58</sub>O<sub>12</sub> + Na 753.3826. Found: 753.3808.

Synthesis of B1794:



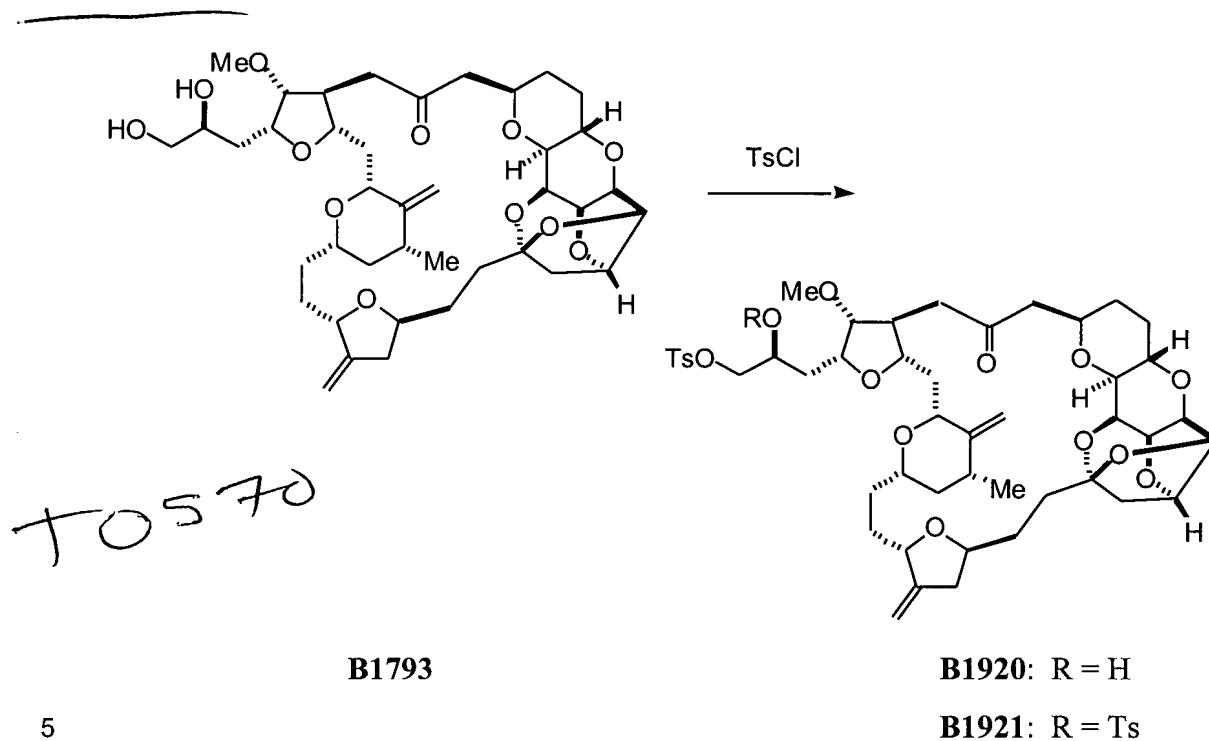
5

Arabinose

B1794

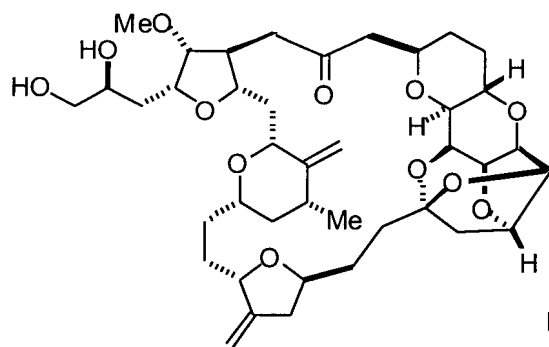
**B1794** Except for stereochemical and protecting group differences (Schemes 3 and 5), arabinose was converted to **B1794** in a manner similar to that described for **B1793** (see schemes 10 4 and 5). HRMS: calcd for  $C_{40}H_{58}O_{12} + Na$  753.3826. Found: 753.3856.

# Synthesis of Representative B1793 Analogs:

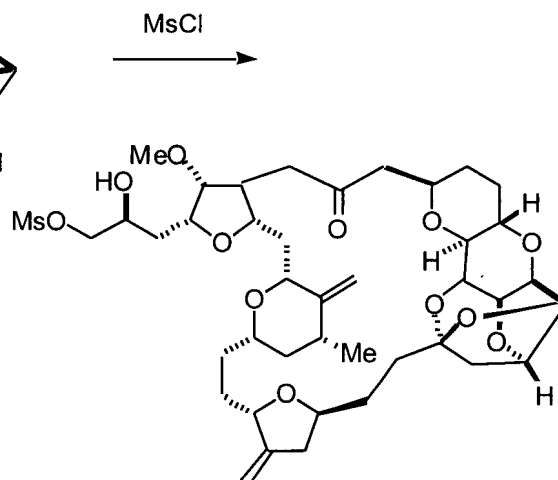


- B1920 and B1921** TsCl (9.9 mg, 0.052 mmol) was added to a solution of diol **B1793** (7.6 mg, 0.010 mmol) in  $\text{CH}_2\text{Cl}_2$  (1 mL) and pyridine (0.1 mL) at rt. After 48 h, the reaction was quenched with a 1:4 mixture of saturated aqueous  $\text{NaHCO}_3$ -brine and extracted with  $\text{CH}_2\text{Cl}_2$  (4 $\times$ ).
- 10 The combined extracts were dried over  $\text{Na}_2\text{SO}_4$  and concentrated. Purification by preparative TLC (80% EtOAc-hexanes) afforded monotosylate **B1920** (6.0 mg, 67%), and ditosylate **B1921** (1.8 mg, 18%).



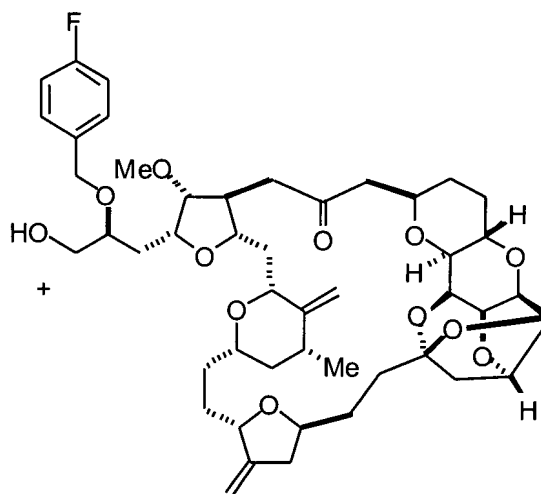
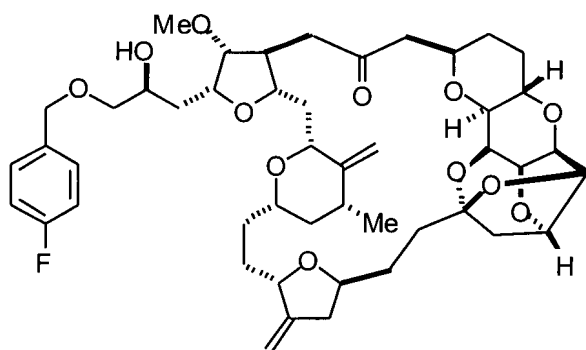
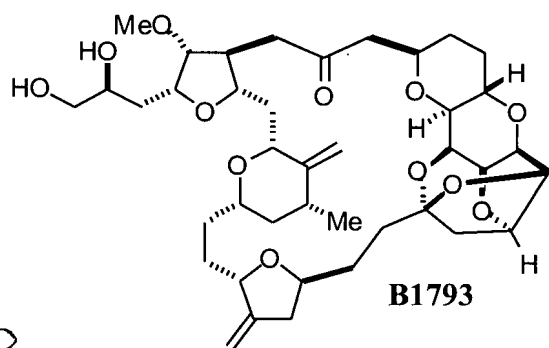


**B1793**



**B2294**

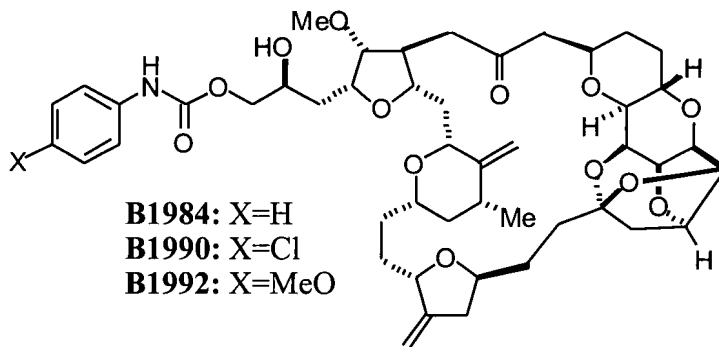
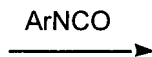
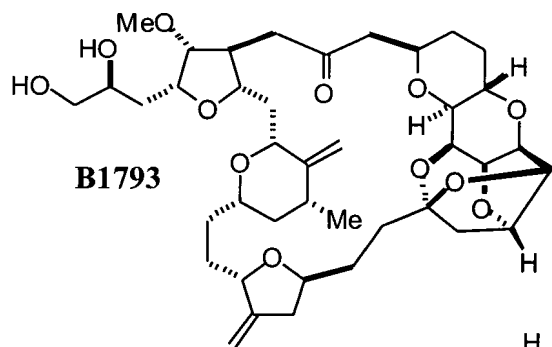
**B2294** MsCl (0.3 M in CH<sub>2</sub>Cl<sub>2</sub>, 98 μL, 0.030 mmol) was added dropwise over 40 min to a mixture of collidine (7 μL, 0.054 mmol), **B1793** (20.8 mg, 0.028 mmol) and CH<sub>2</sub>Cl<sub>2</sub> (1 mL) at 0 °C. After 76 h at 4 °C, the reaction was quenched with a 1:4 mixture of saturated aqueous NaHCO<sub>3</sub>-brine and extracted with CH<sub>2</sub>Cl<sub>2</sub> (4×). The combined extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The crude product was dissolved in toluene (3 mL) concentrated and purified by preparative TLC (1.5% MeOH-EtOAc) to afford mesylate **B2294** (21.4 mg, 95%).



**B2014 and B2015** A 0.016 M solution of 4-fluorobenzyl bromide in Et<sub>2</sub>O (800 μL, 13

5 μmol) and Ag<sub>2</sub>O (10 mg, 43 μmol) were each added in three portions at 1 h intervals to a rt solution of **B1793** (1.7 mg, 2.3 μmol) in Et<sub>2</sub>O (1.2 mL). The mixture was protected from light, stirred for 7 h and then filtered through Celite. Concentration and purification by preparative TLC (EtOAc) afforded primary ether **B2014** (1.1 mg, 56%), and secondary ether **B2015** (0.6 mg, 31%). HRMS (FAB): calcd for C<sub>47</sub>H<sub>63</sub>FO<sub>12</sub> + Na 861.4201. Found: for **B2014** 861.4178, for

10 **B2015** 861.4160.

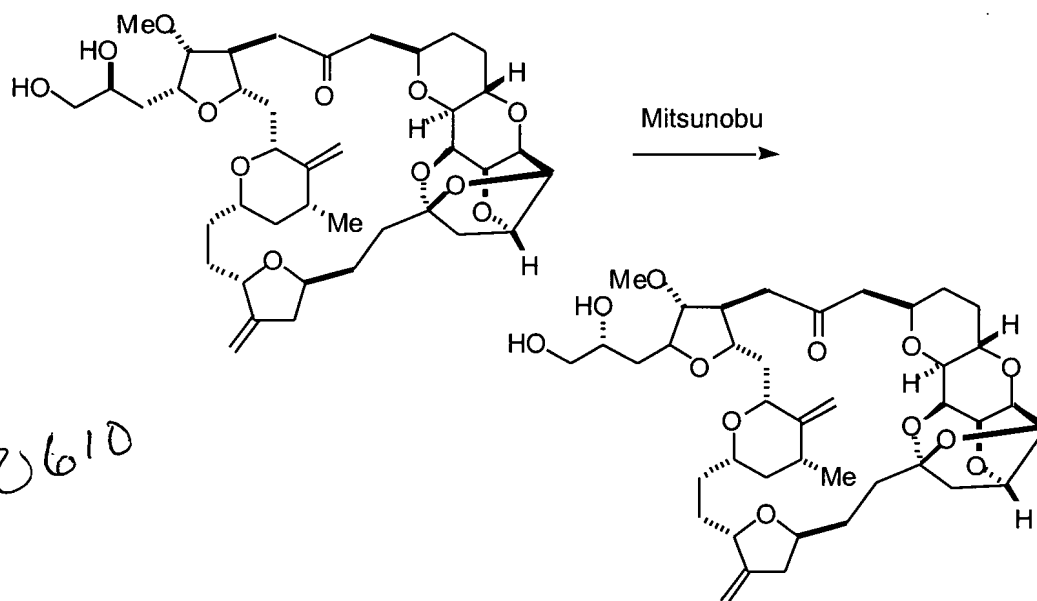


**General** A mixture of **B1793** (1 mg, 1.37 micromol), Et<sub>3</sub>N (10 microL, 72 micromol) and ArNCO (2 to 4 equiv.) in CH<sub>2</sub>Cl<sub>2</sub> (0.2 mL) was stirred at rt for 4 h to overnight until the reaction was judged to be complete by TLC. The reaction mixture was diluted with saturated NaHCO<sub>3</sub> (3 mL), extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×) and EtOAc (2×), dried over Na<sub>2</sub>SO<sub>4</sub> and purified by preparative TLC (5% MeOH-CH<sub>2</sub>Cl<sub>2</sub>) to afford the products:

**B1984** (1.0 mg, 86%) HRMS (FAB): calcd for C<sub>47</sub>H<sub>63</sub>NO<sub>13</sub> + Na 872.4197. Found: 872.4214.

**B1990** (1.1 mg, 92%) HRMS (FAB): calcd for C<sub>47</sub>H<sub>62</sub>ClNO<sub>13</sub> + Na 906.3807. Found: 906.3826.

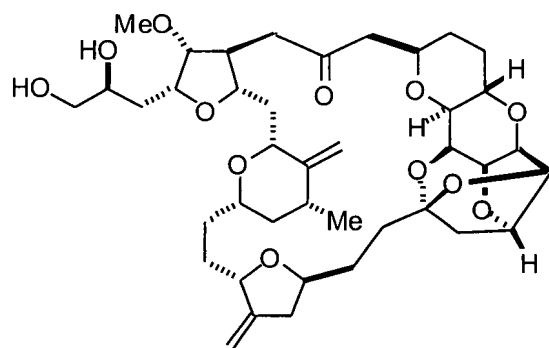
**B1992** (1.0 mg, 83%) HRMS (FAB): calcd for C<sub>48</sub>H<sub>65</sub>NO<sub>14</sub> + Na 902.4303. Found: 902.4269.



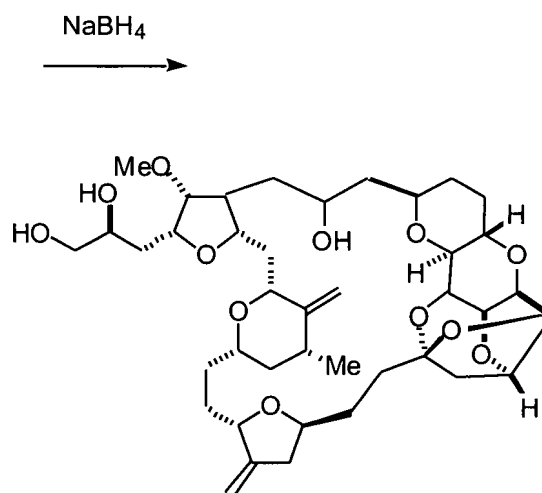
**B1793**

**B2042**

**B2042** DEAD (0.4 M in ether, 50  $\mu$ L, 19  $\mu$ mol) was added to a solution of **B1793** (2.0 mg, 2.7  $\mu$ mol), triphenylphosphine (5 mg, 19  $\mu$ mol), 4-nitrobenzoic acid (3.2 mg, 19  $\mu$ mol) and Et<sub>2</sub>O (500  $\mu$ L) at rt. After 22 h, the reaction mixture was loaded directly onto a preparative TLC plate and eluted with 60% EtOAc-hexanes to give the intermediate diester (3.0 mg). This material was taken up in MeOH (300  $\mu$ L) and treated with K<sub>2</sub>CO<sub>3</sub> (approximately 1 mg). After stirring at rt for 30 min, the reaction mixture was diluted with brine and extracted with CH<sub>2</sub>Cl<sub>2</sub> (5 $\times$ ). The combined extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by preparative TLC (5% MeOH-EtOAc) to afford **B2042** (1.2 mg, 60% for two steps). HRMS (FAB): calcd for C<sub>40</sub>H<sub>58</sub>O<sub>12</sub> + Na 753.3826. Found: 753.3810.

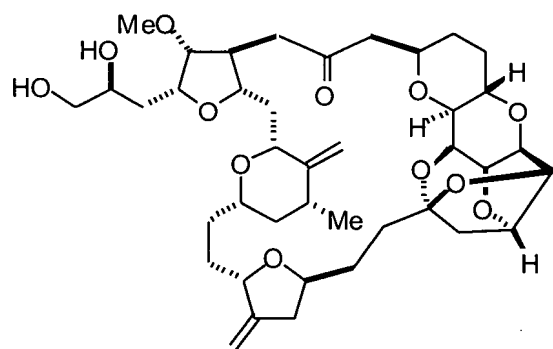


**B1793**

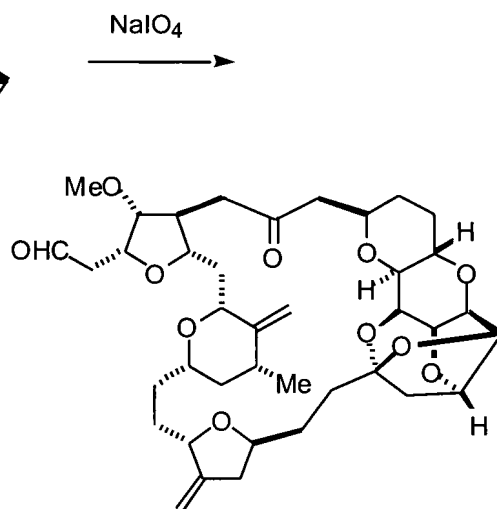


**B1896, B1897**

**B1896** and **B1897** NaBH<sub>4</sub> (3 mg, 0.08 mmol) was added to a solution of **B1793** (2.30 mg, 3.15 μmol) in 1:1 CH<sub>2</sub>Cl<sub>2</sub>-MeOH (0.2 mL) at rt. Concentration of the reaction mixture and purification by preparative TLC (8% MeOH-EtOAc) provided **B1896** (0.80 mg, 35%) and **B1897** (2:1 mixture, 0.15 mg, 6.5%). HRMS (FAB) for **B1896**: calcd for C<sub>40</sub>H<sub>60</sub>O<sub>12</sub> + Na 755.3983. Found: 753.3969.

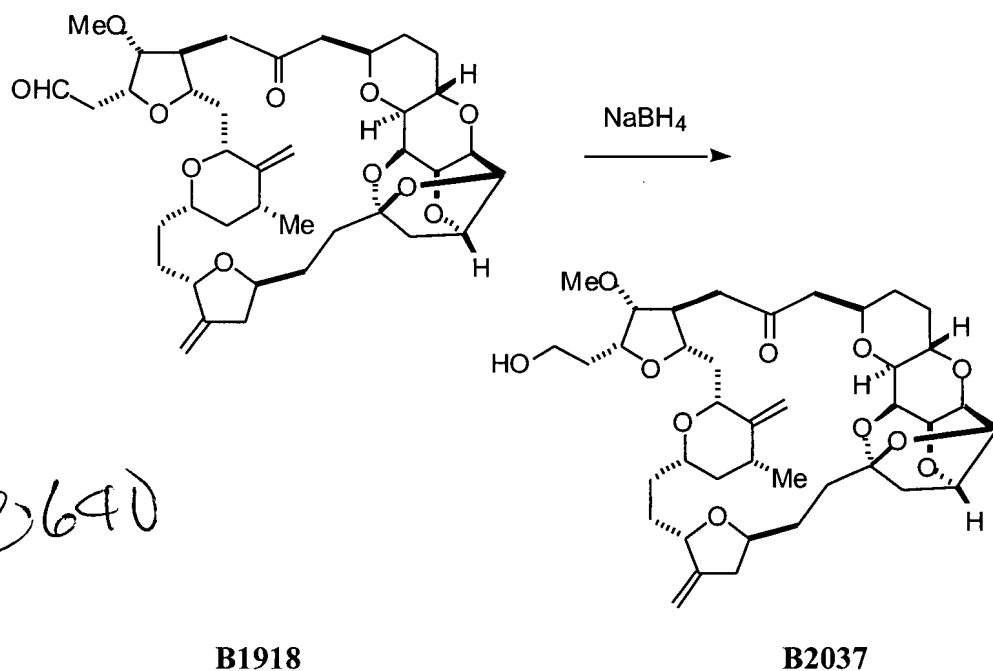


**B1793**

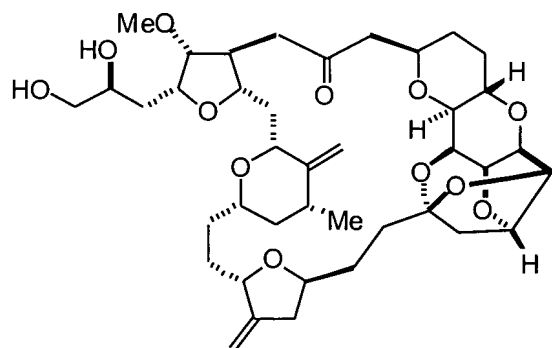


**B1918**

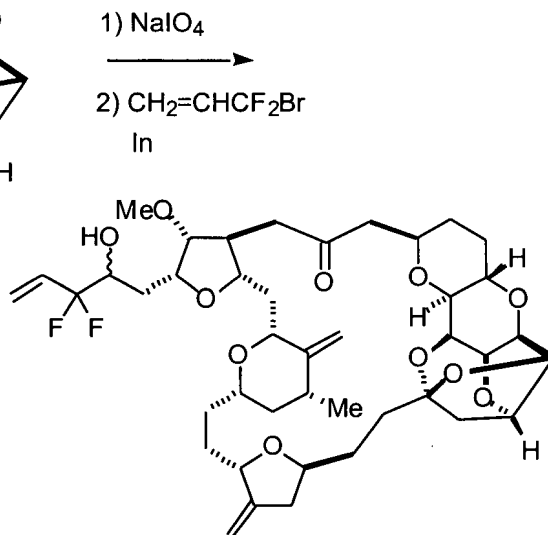
**B1918** A mixture of **B1793** (2.0 mg, 2.74  $\mu$ mol),  $\text{NaIO}_4$  (35 mg, 0.16 mmol), MeOH (0.8 mL) and  $\text{H}_2\text{O}$  (0.2 mL) was stirred at rt for 40 min. The reaction mixture was diluted with  $\text{H}_2\text{O}$  (1 mL), extracted with  $\text{CH}_2\text{Cl}_2$  (6 $\times$ ), dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by column chromatography (5% MeOH- $\text{CH}_2\text{Cl}_2$ ) to give **B1918** (1.9 mg, 100%).



**B2037** A 0.034 M solution of  $\text{NaBH}_4$  (0.1 mL, 3.4  $\mu\text{mol}$ ) in EtOH was added portion-  
 wise to a solution of **B1918** (1.9 mg, 2.72  $\mu\text{mol}$ ) in MeOH (0.8 mL) and  $\text{CH}_2\text{Cl}_2$  (0.2 mL) at  $-78^\circ\text{C}$  to rt until the reaction was judged to be complete by TLC. The reaction was quenched with saturated aqueous  $\text{NH}_4\text{Cl}$  (2 mL) at  $-78^\circ\text{C}$ , warmed to rt, extracted with  $\text{CH}_2\text{Cl}_2$  (6 $\times$ ), dried over  $\text{Na}_2\text{SO}_4$  and purified by preparative TLC (5% MeOH-  $\text{CH}_2\text{Cl}_2$ ) to afford **B2037** (1.7 mg, 89%).  
 HRMS (FAB): calcd for  $\text{C}_{39}\text{H}_{56}\text{O}_{11} + \text{Na}$  723.3720. Found: 723.3749.



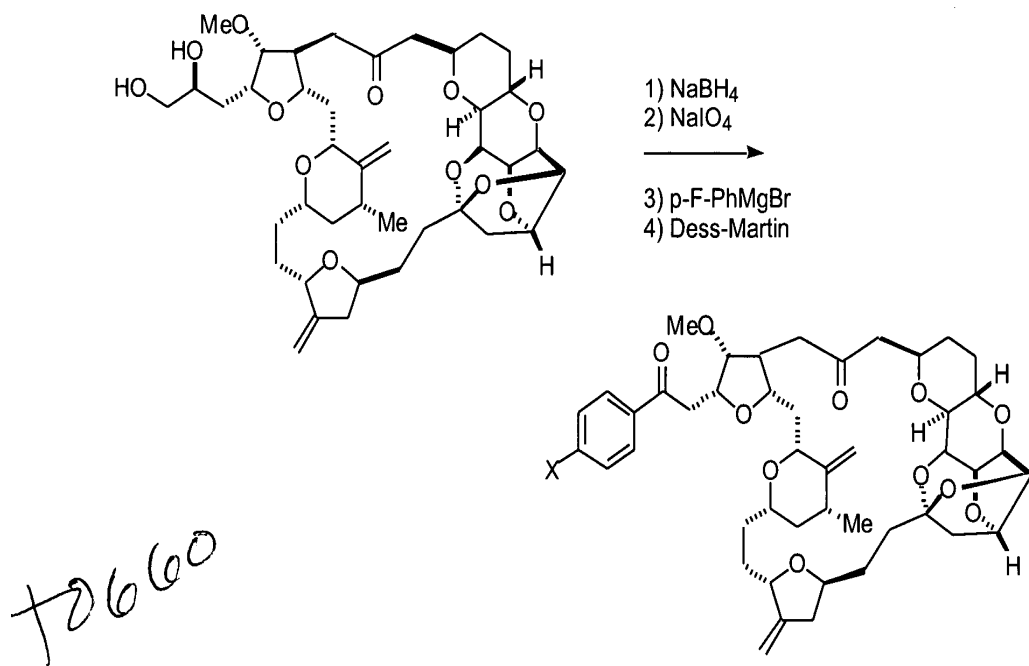
**B1793**



**B2035**

- 5 **B2035** NaIO<sub>4</sub> (35 mg, 0.16 mmol) was added to a solution of **B1793** (1.7 mg, 0.0023 mmol), MeOH (800 μL) and H<sub>2</sub>O (200 μL) and after 15 min, the mixture was diluted with H<sub>2</sub>O and extracted with CH<sub>2</sub>Cl<sub>2</sub> (5×). The organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and the intermediate aldehyde was immediately dissolved in DMF (300 μL). 3-Bromo-3,3-difluoropropene (3 μL, 0.023 mmol) and indium powder (3 mg, 0.23 mmol) were added and after
- 10 24 h additional 3-bromo-3,3-difluoropropene (1 μL, 0.008 mmol) was added. After 18 h, H<sub>2</sub>O was added the mixture was extracted with EtOAc (3×). The combined organic extracts were washed successively with H<sub>2</sub>O and brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by preparative TLC (80% EtOAc-hexanes) to provide **B2035** (0.74 mg, 41% for 2 steps) as a mixture of isomers. HRMS (FAB): calcd for C<sub>42</sub>H<sub>58</sub>F<sub>2</sub>O<sub>11</sub> + Na 799.3845. Found: 799.3883.





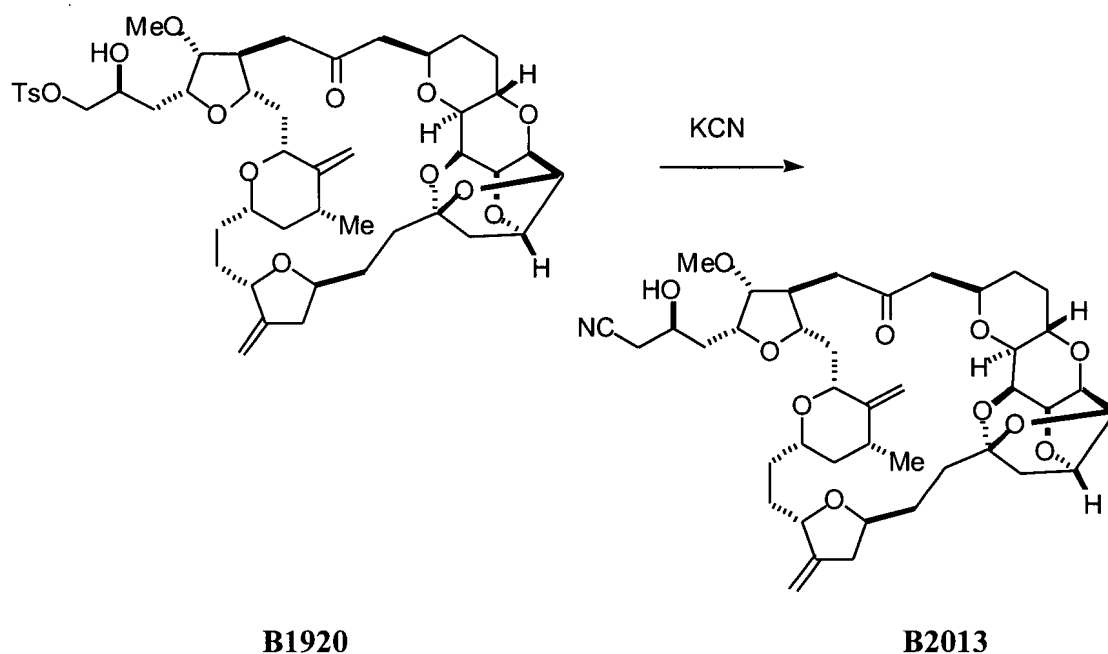
5 — **B2008, B2011** NaBH<sub>4</sub> (2 mg, 0.05 mmol) was added to a solution of **B1793** (2.2 mg, 0.003 mmol) in 1:1 CH<sub>2</sub>Cl<sub>2</sub>-MeOH (200 μL) at rt. After 15 min saturated aqueous NH<sub>4</sub>Cl and H<sub>2</sub>O were added, and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (6×) and EtOAc (2×). The combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (10% MeOH-EtOAc) to provide an intermediate triol, which was dissolved in

10 MeOH (800 μL) and H<sub>2</sub>O (200 μL). NaIO<sub>4</sub> (35 mg, 0.16 mmol) was added and after 20 min, the mixture was diluted with H<sub>2</sub>O and extracted with CH<sub>2</sub>Cl<sub>2</sub> (6×). The organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and the intermediate aldehyde was immediately dissolved in THF (500 μL). 4-Fluorophenylmagnesium bromide (2M in Et<sub>2</sub>O, 12 μL, 0.024 mmol) was added and after 20 min the reaction was quenched with saturated aqueous NH<sub>4</sub>Cl. The mixture was

15 extracted with CH<sub>2</sub>Cl<sub>2</sub> (6×) and the combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification by preparative TLC (EtOAc) provided the desired product as a mixture of 4 isomers (1.32 mg, 55% for 3 steps).

Dess-Martin periodinane (~3 mg, 0.007 mmol) was added to a solution of the above product (0.95 mg, 0.0012 mmol) in  $\text{CH}_2\text{Cl}_2$  (300  $\mu\text{L}$ ) and the mixture was stirred at rt for 20 min. Additional Dess-Martin periodinane (~3 mg, 0.007 mmol) and  $\text{CH}_2\text{Cl}_2$  (300  $\mu\text{L}$ ) were added and after another 40 min  $\text{Et}_2\text{O}$ , saturated aqueous  $\text{NaHCO}_3$  (4 mL) and saturated aqueous  $\text{Na}_2\text{S}_2\text{O}_3$  (1 mL) were added. The mixture was extracted with  $\text{Et}_2\text{O}$  (3 $\times$ ) and the combined extracts were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide **B2008** (0.58 mg, 61%). HRMS (FAB): calcd for  $\text{C}_{45}\text{H}_{57}\text{FO}_{11} + \text{Na}$  815.3783. Found: 815.3758.

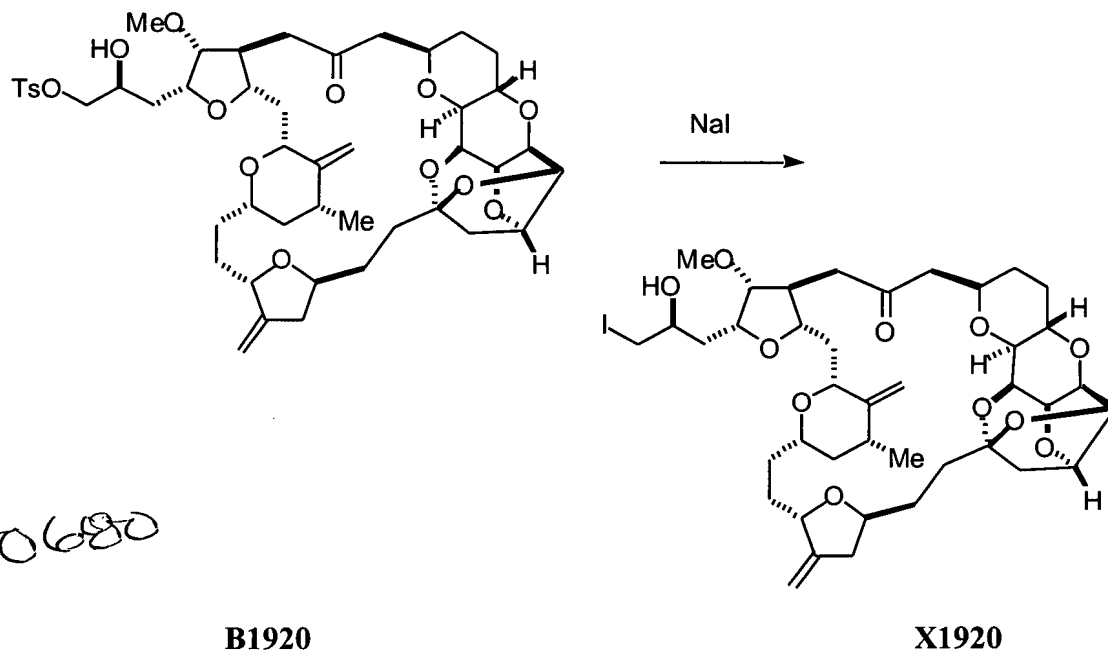
**B2011** In an analogous manner, **B1793** (1.9 mg, 0.003 mmol) was converted to **B2011** (0.87 mg, 42% for 4 steps). HRMS (FAB): calcd for  $\text{C}_{45}\text{H}_{58}\text{O}_{11} + \text{Na}$  797.3877. Found: 797.3877.



**B2013** A solution of **B1920** (1.4 mg, 0.0016 mmol),  $\text{KCN}$  (1 mg, 0.016 mmol) and DMSO (500  $\mu\text{L}$ ) was heated at 60  $^\circ\text{C}$  for 8 h. After cooling to rt,  $\text{H}_2\text{O}$  was added and the mixture was extracted with EtOAc (3 $\times$ ). The combined organic extracts were washed

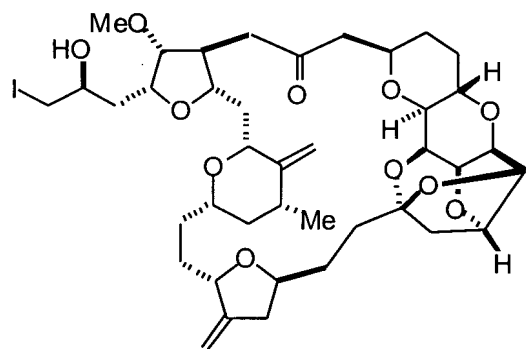
successively with H<sub>2</sub>O and brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by preparative TLC (80% EtOAc-hexanes) to provide **B2013** (0.78 mg, 67%). HRMS (FAB): calcd for C<sub>41</sub>H<sub>57</sub>NO<sub>11</sub> + Na 762.3829. Found: 762.3848.

5

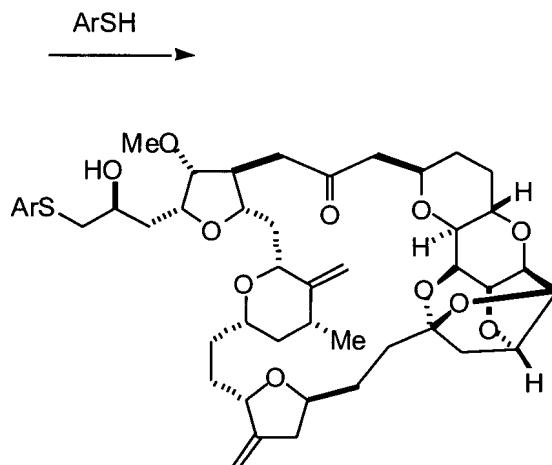


**X1920** A mixture of **B1920** (1.3 mg, 1.47  $\mu$ mol), NaI (30 mg, excess) and acetone (1 mL) was stirred at 60 °C for 3.5 h. After cooling to rt, the reaction mixture was diluted with saturated aqueous NaHCO<sub>3</sub> (3 mL), extracted with CH<sub>2</sub>Cl<sub>2</sub> (5 $\times$ ) and EtOAc, dried over Na<sub>2</sub>SO<sub>4</sub> and purified by column chromatography (50% EtOAc-CH<sub>2</sub>Cl<sub>2</sub> to 80% EtOAc-hexanes) to give the iodide **X1920** (1.3 mg, 100%).

15



**X1920**



**B1998:** Ar = *p*-Cl-Ph

**B2010:** Ar = *p*-MeO-Ph

**B2019:** Ar = 2-imidazole

5

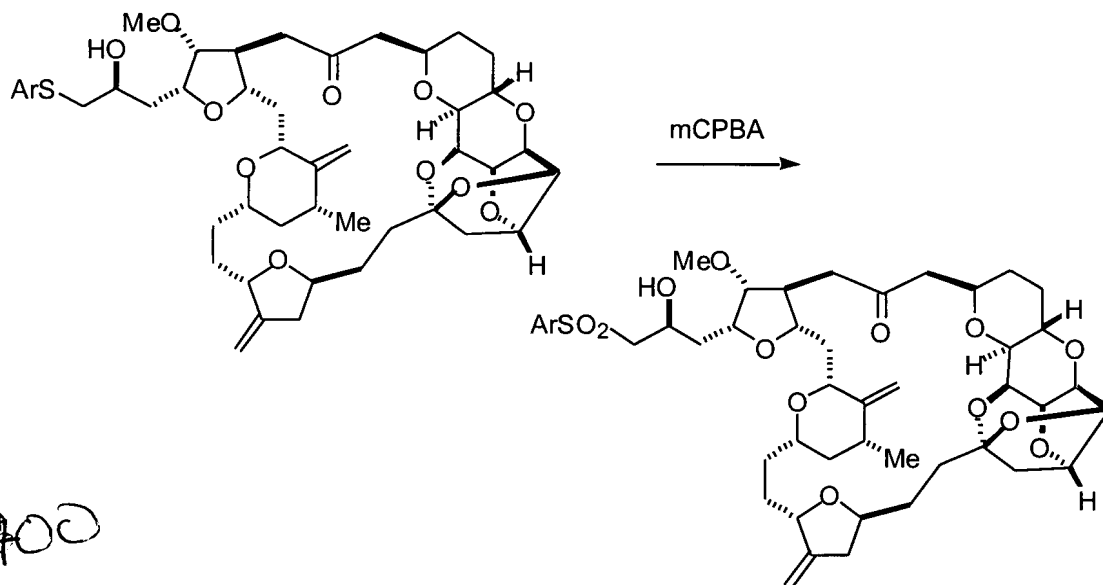
**General** A mixture of iodide **X1920** (1.0 equiv.),  $i\text{Pr}_2\text{EtN}$  (11 to 22 equiv.), ArSH (9 to 46 equiv.) and DMF (0.3 mL) was stirred at rt until the reaction was judged to be complete by TLC (typically 24 to 48 h). The reaction mixture was diluted with saturated aqueous  $\text{NaHCO}_3$  (2 mL), extracted with  $\text{CH}_2\text{Cl}_2$  and EtOAc, dried over  $\text{Na}_2\text{SO}_4$  and purified by preparative TLC (80% EtOAc-hexanes or 5% MeOH- $\text{CH}_2\text{Cl}_2$ ) to afford the sulfide products:

**B1998** (1.3 mg gave 1.1 mg, 85%) HRMS (FAB): calcd for  $\text{C}_{46}\text{H}_{61}\text{ClO}_{11}\text{S} + \text{Na}$  897.3521. Found: 897.3533.

**B2010** (1.1 mg gave 0.7 mg, 59%). HRMS (FAB): calcd for  $\text{C}_{47}\text{H}_{64}\text{O}_{12}\text{S} + \text{Na}$  875.4016. Found: 875.4057.

**B2019** (1.1 mg gave 0.7 mg, 61%) MS (FAB): M + Na

15



**B1998:** Ar = *p*-Cl-Ph

**B2010:** Ar = *p*-MeO-Ph

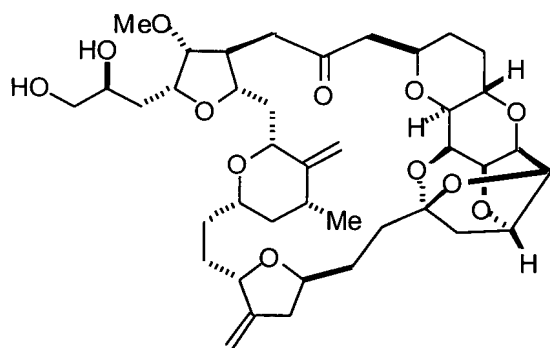
**B2016:** Ar = *p*-Cl-Ph

**B2030:** Ar = *p*-MeO-Ph

5      **General** A 0.01 M solution of mCPBA (1.2 equiv.) in CH<sub>2</sub>Cl<sub>2</sub> was added to a solution of a sulfide (1.0 equiv.) in CH<sub>2</sub>Cl<sub>2</sub> (0.5 mL) at 0 °C for 30 min. The reaction mixture was diluted with saturated NaHCO<sub>3</sub> (2 mL), extracted with CH<sub>2</sub>Cl<sub>2</sub> and EtOAc, dried over Na<sub>2</sub>SO<sub>4</sub> and purified by preparative TLC (80% EtOAc-hexanes or EtOAc) to afford the products:

**B2016** (0.9 mg gave 0.7 mg, 74%)

10      **B2030** (1.0 mg gave 0.6 mg, 61%) HRMS (FAB): calcd for C<sub>47</sub>H<sub>64</sub>O<sub>14</sub>S + Na 907.3914.  
Found: 907.3950.

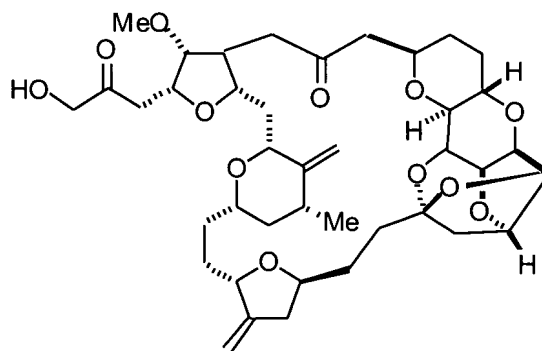


**B1793**

1) TBDPSCI

2) Dess-Martin

3) TBAF



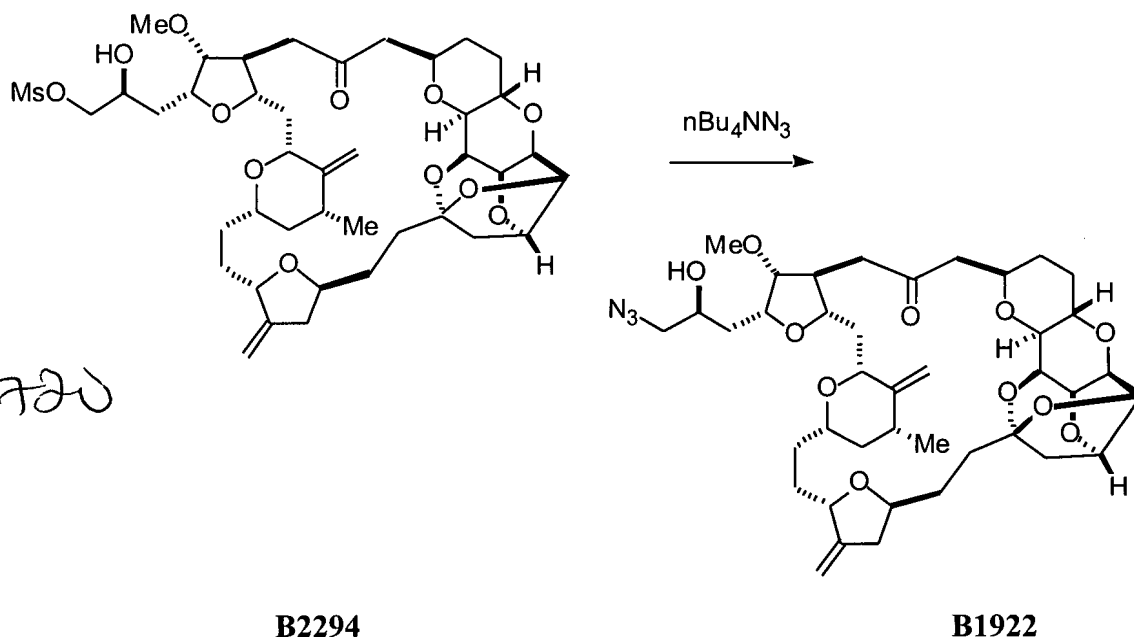
**B1934**

**B1934** TBDPSCI (3.0  $\mu$ L, 12  $\mu$ mol) was added to a solution of **B1793** (1.3 mg, 1.78

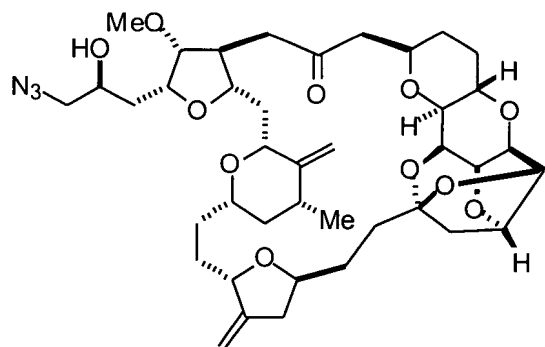
5  $\mu$ mol), imidazole (10 mg, 166  $\mu$ mol) and DMF (0.10 mL) at rt. After stirring for 1 h, the reaction mixture was diluted with saturated aqueous  $\text{NaHCO}_3$  (2 mL), extracted with  $\text{CH}_2\text{Cl}_2$  (3 $\times$ ) and EtOAc (2 $\times$ ), dried over  $\text{Na}_2\text{SO}_4$  and purified by preparative TLC (5% MeOH- $\text{CH}_2\text{Cl}_2$ ) to give the intermediate silyl ether (1.3 mg, 77%).

This material was dissolved in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) and treated with Dess-Martin  
 10 periodinane (10 mg, 24  $\mu$ mol) for 1.5 h at rt, diluted with  $\text{Et}_2\text{O}$  and filtered through Celite. The filtrate was concentrated and purified by preparative TLC (50% EtOAc-hexanes) to afford the diketone intermediate (1.0 mg, 77%), which was dissolved in THF (0.5 mL) and treated with 0.02 M TBAF containing 0.01 M imidazole hydrochloride (THF solution, 75  $\mu$ L, 1.5  $\mu$ mol) at rt for 15 min. The reaction mixture was eluted through a  $\text{SiO}_2$  column (50% EtOAc-hexanes to 5%  
 15 MeOH- $\text{CH}_2\text{Cl}_2$ ) and the desired product was further purified by preparative TLC (5% MeOH- $\text{CH}_2\text{Cl}_2$ ) to afford **B1934** (0.75 mg, 100%). HRMS (FAB): calcd for  $\text{C}_{40}\text{H}_{56}\text{O}_{12} + \text{Na}$  751.3669. Found: 751.3690.

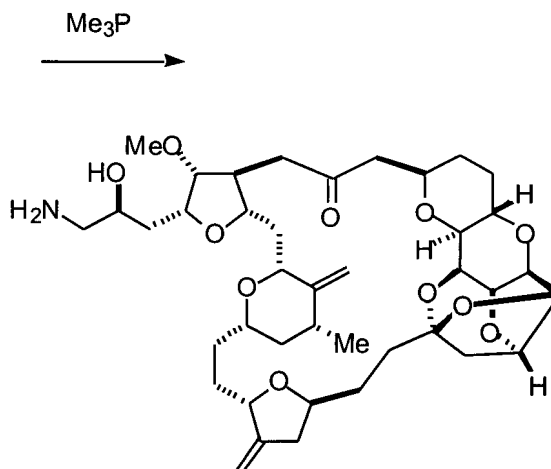
Synthesis of B1939:



**B1922** Tetra-n-butylammonium azide (0.2 M in DMF, 0.5 mL, 0.10 mmol) was added to a solution of mesylate **B2294** (21.4 mg, 0.026 mmol) in DMF (2 mL) at rt. After stirring at 83 °C for 3.5 h, the reaction mixture was cooled to rt, diluted with toluene, concentrated and purified by preparative TLC (80% ethyl acetate-hexanes) to furnish **B1922** (18 mg, 92%).



**B1922**



**B1939**

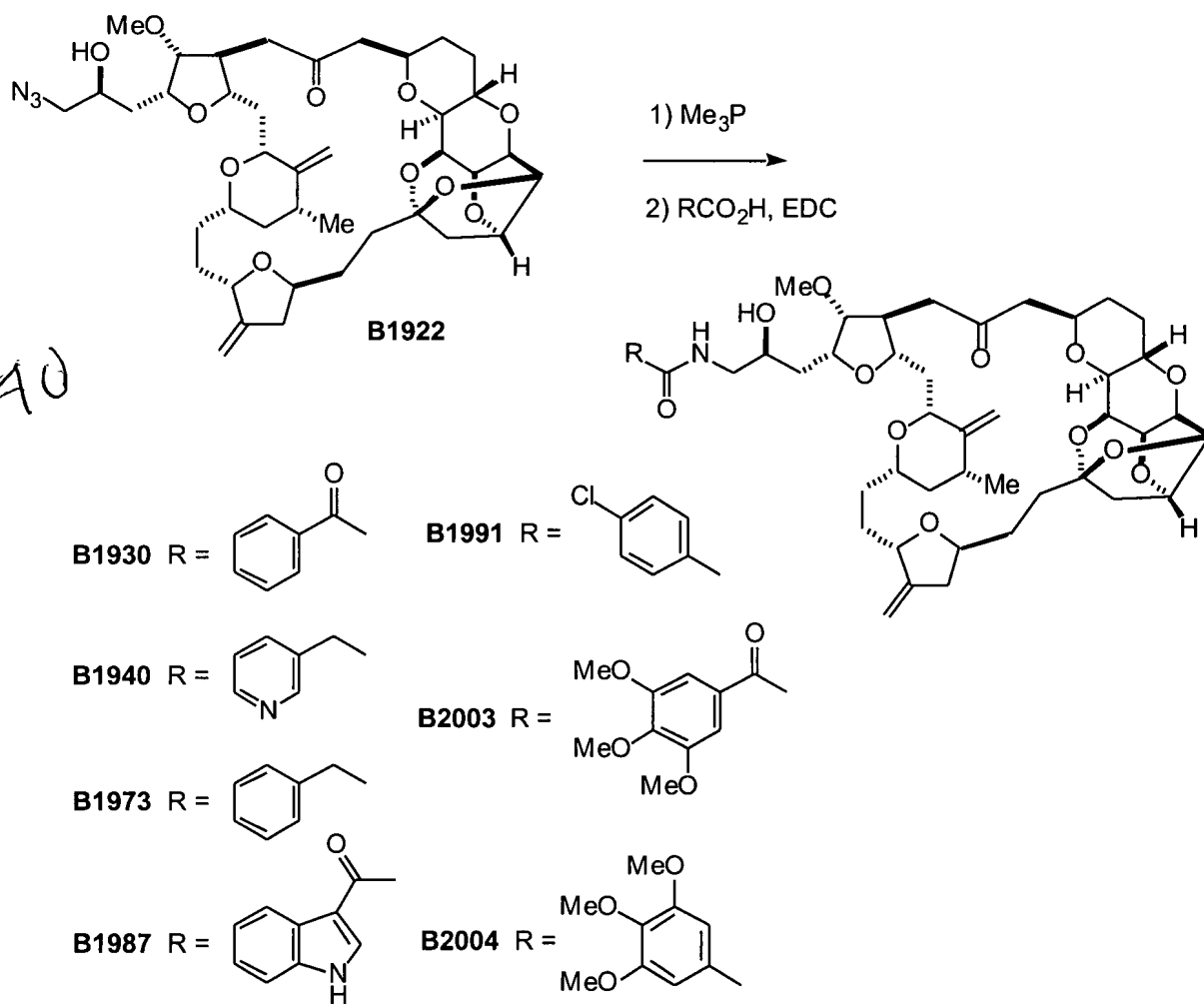
**B1939** Me<sub>3</sub>P (1 M in THF) and H<sub>2</sub>O (0.8 mL) were sequentially added to a solution of  
 5 azide **B1922** (24.6 mg, 0.032 mmol) in THF (3.2 mL) at rt. The mixture was stirred for 22 h,  
 diluted with toluene, concentrated and purified by flash chromatography [step gradient, 10%  
 MeOH-EtOAc followed by MeOH-EtOAc-30% aqueous NH<sub>4</sub>OH (9:86:5)] to provide the desired  
 primary amine (23.3 mg), which by <sup>1</sup>H-NMR contained ~1% trimethylphosphine oxide.

Lyophilization from benzene and standing under high vacuum for 2 d furnished **B1939** (20.3 mg,  
 10 87%).



## Synthesis of Representative B1939 Analogs:

*B1930, B1940, B1973, B1987, B1988, B1991, B2003, B2004*



5

10

**B1930**  $\text{Me}_3\text{P}$  (1 M in THF, 13  $\mu\text{L}$ , 0.013 mmol) was added to a solution of **B1922** (1.6 mg, 2.1  $\mu\text{mol}$ ), THF (400  $\mu\text{L}$ ) and  $\text{H}_2\text{O}$  (100  $\mu\text{L}$ ) at rt. The mixture was stirred for 22 h, diluted with toluene, concentrated, and azeotropically dried with toluene (2 $\times$ ) to give the crude amine which was used directly in the next step.

EDC (0.06 M in  $\text{CH}_2\text{Cl}_2$ , 100  $\mu\text{L}$ , 11  $\mu\text{mol}$ ) was added to a solution of the crude amine, benzoylformic acid (0.8 mg, 5.3  $\mu\text{mol}$ ) and  $\text{CH}_2\text{Cl}_2$  (200  $\mu\text{L}$ ) at rt. After 30 min, the reaction was

quenched with a 1:4 mixture of saturated aqueous  $\text{NaHCO}_3$ -brine and extracted with  $\text{CH}_2\text{Cl}_2$  (5 $\times$ ). The combined extracts were dried over  $\text{Na}_2\text{SO}_4$  and concentrated and purified by preparative TLC (EtOAc) to afford **B1930** (1.5 mg, 83% for two steps). HRMS (FAB): calcd for  $\text{C}_{48}\text{H}_{63}\text{NO}_{13} + \text{Na}$  884.4197. Found: 884.4166.

5        **B1940** Using the procedure described above for **B1930**, **B1922** was reduced, coupled with 3-pyridylacetic acid hydrochloride and purified by preparative TLC [(MeOH-EtOAc-30% aqueous  $\text{NH}_4\text{OH}$  (9:86:5))] to afford **B1940** (0.8 mg, 67 % for two steps). HRMS (FAB): calcd for  $\text{C}_{47}\text{H}_{64}\text{N}_2\text{O}_{12} + \text{Na}$  871.4357. Found: 871.4362.

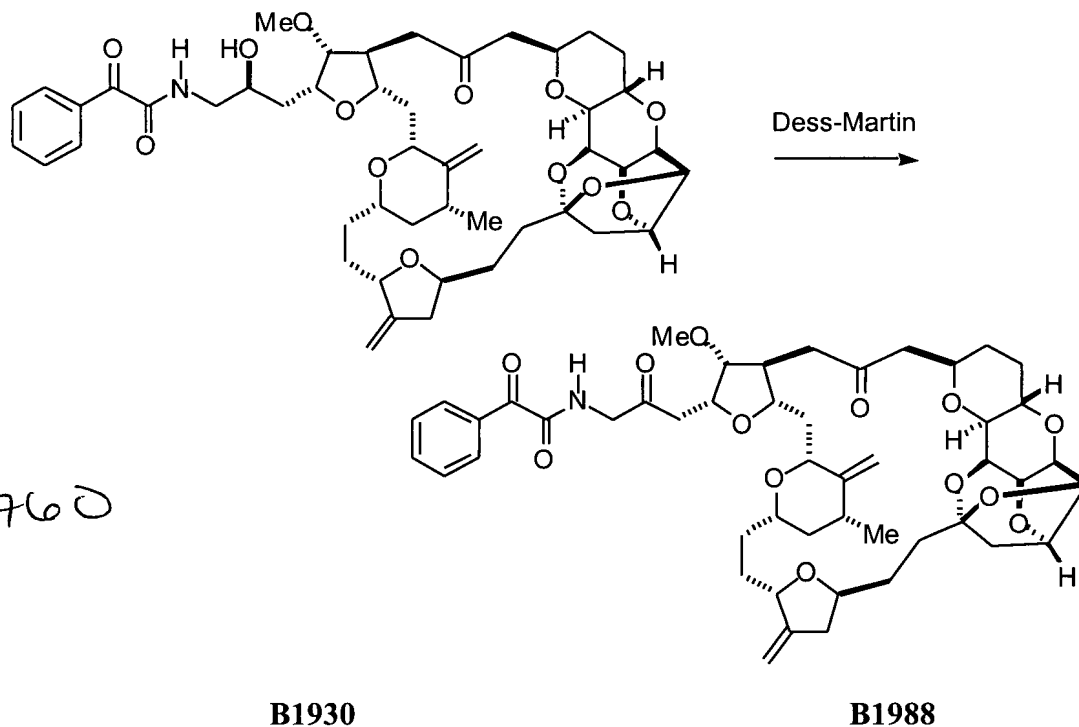
10        **B1973** Using the procedure described above, **B1922** (0.9 mg, 1.2  $\mu\text{mol}$ ) was reduced, coupled with phenylacetic acid and purified by preparative TLC (5% MeOH-EtOAc) to afford **B1973** (0.44 mg, 44 % for two steps). HRMS (FAB): calcd for  $\text{C}_{48}\text{H}_{65}\text{NO}_{12} + \text{Na}$  870.4404. Found: 870.4447.

15        **B1987** Using the procedure described above, **B1922** (0.9 mg, 1.2  $\mu\text{mol}$ ) was reduced, coupled with 3-indoleglyoxylic acid and purified by preparative TLC (3% MeOH-EtOAc) to afford **B1987** (0.8 mg, 75 % for two steps). HRMS (FAB): calcd for  $\text{C}_{50}\text{H}_{64}\text{N}_2\text{O}_{12} + \text{Na}$  923.4306. Found: 923.4338.

20        **B1991** Using the procedure described above, **B1922** (1.0 mg, 1.3  $\mu\text{mol}$ ) was reduced, coupled with 4-chlorobenzoic acid and purified by preparative TLC (3% MeOH-EtOAc) to afford **B1991** (0.8 mg, 70 % for two steps). HRMS (FAB): calcd for  $\text{C}_{47}\text{H}_{62}\text{ClNO}_{12} + \text{Na}$  890.3858. Found: 890.3843.

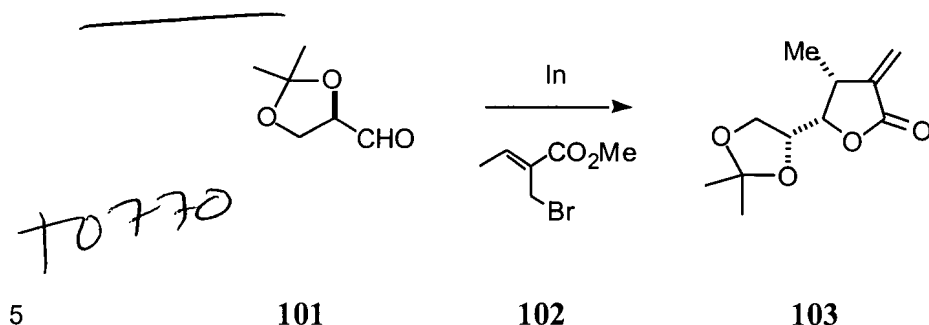
**B2003** Using the procedure described above, **B1922** (1.0 mg, 1.3  $\mu\text{mol}$ ) was reduced, coupled with 3,4,5-trimethoxybenzoylformic acid and purified by preparative TLC (EtOAc) to afford **B2003** (0.7 mg, 56 % for two steps). HRMS (FAB): calcd for  $\text{C}_{51}\text{H}_{69}\text{NO}_{16} + \text{Na}$  974.4514. Found: 974.4525.

25        **B2004** Using the procedure described above, **B1922** (1.0 mg, 1.3  $\mu\text{mol}$ ) was reduced, coupled with 3,4,5-trimethoxybenzoic acid and purified by preparative TLC (5% MeOH-EtOAc) to afford **B2004** (0.7 mg, 58 % for two steps). HRMS (FAB): calcd for  $\text{C}_{49}\text{H}_{65}\text{NO}_{13} + \text{Na}$  946.4565. Found: 946.4599.

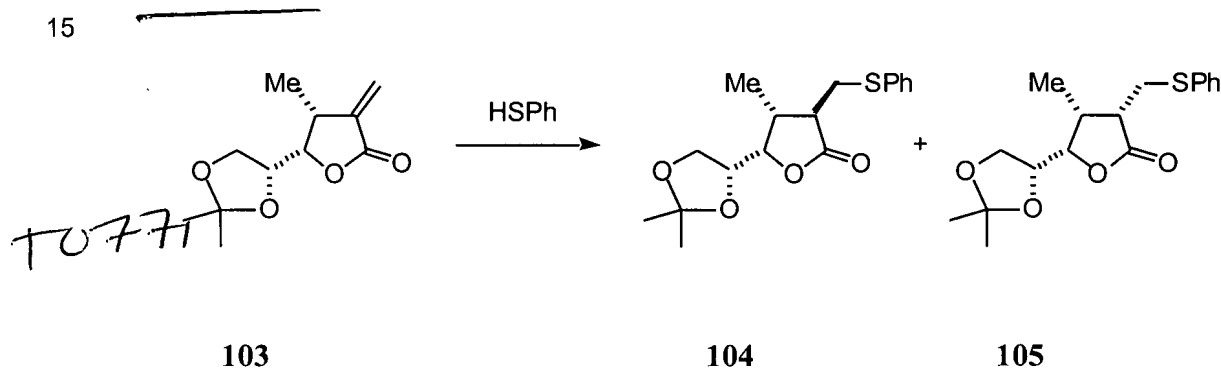


**B1988** Dess-Martin periodinane (1 mg, 2.3  $\mu\text{mol}$ ) was added to a solution of **B1930** (0.80 mg, 0.93  $\mu\text{mol}$ ) in  $\text{CH}_2\text{Cl}_2$  (500  $\mu\text{L}$ ) at rt. After 1 h, the reaction was diluted with  $\text{Et}_2\text{O}$  and filtered through Celite. The filtrate was washed sequentially with a 1:9 mixture of saturated aqueous  $\text{NaHCO}_3$ - $\text{Na}_2\text{S}_2\text{O}_3$  and brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by preparative TLC (80%  $\text{EtOAc}$ -hexanes) to afford **B1988** (0.45 mg, 56%). HRMS (FAB): calcd for  $\text{C}_{48}\text{H}_{61}\text{NO}_{13} + \text{Na}$  882.4041. Found: 884.4012.

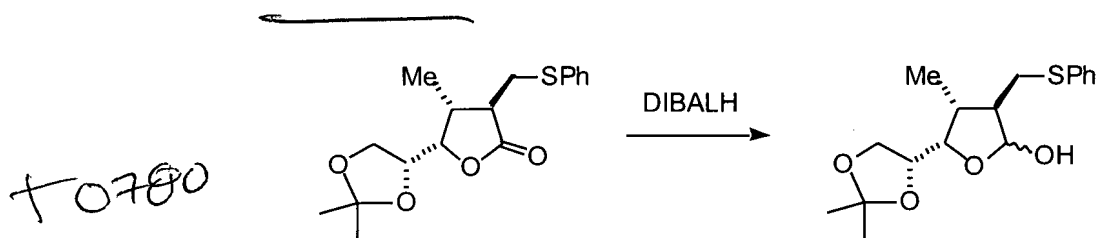
**Synthesis of B2090:**



**Compound 103** Indium powder (1.35 g, 11.8 mmol) was added to a solution of **102** (3.38 g, 17.6 mmol) in DMF (20 mL) at rt. After stirring for 30 min, the reaction mixture was cooled to 0 °C. Neat aldehyde **101** (3.72 g, 28.6 mmol) was then added and the mixture was stirred overnight while allowing the temperature to warm to rt. The reaction mixture was recooled to 0 °C and then quenched carefully with saturated aqueous NH<sub>4</sub>Cl (100 mL). After stirring for 30 min, the resulting mixture was extracted with Et<sub>2</sub>O (3×), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (10% to 20% EtOAc-hexanes) to give pure crystalline **103** (2.20 g, 59%).



**Compound 104** Et<sub>3</sub>N (72 μL, 0.51 μmol) was added to a solution of **103** (1.09 g, 5.13 mmol) and thiophenol (0.63 mL, 7.16 mmol) in CH<sub>2</sub>Cl<sub>2</sub> and the resulting mixture was stirred at 0 °C for 1 h. Filtration through SiO<sub>2</sub> gave a mixture of **104** and **105**, which after MPLC (15% to 20% EtOAc-hexanes) afforded **104** (0.53 g, 32%) and **105** (0.92 g, 56%).

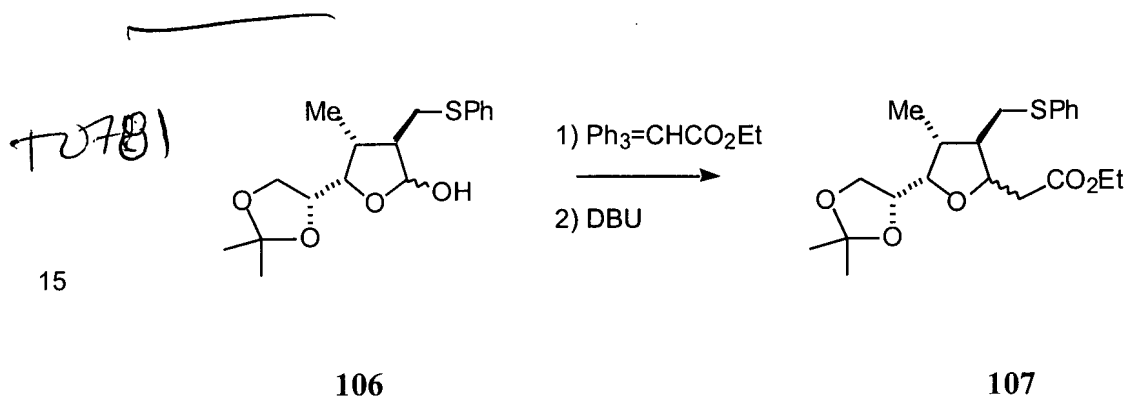


5

104

106

**Compound 106** DIBALH (1 M in toluene, 3.28 mL, 3.28 mmol) was added to a solution of **104** (0.53 g, 1.64 mmol) in toluene (10 mL) at -78 °C and the mixture was stirred at -78°C for 10 min. The reaction was quenched by careful addition of MeOH (0.40 mL, 9.84 mmol) and H<sub>2</sub>O (0.17 mL, 9.84 mmol), warmed to rt and stirred for 20 min. The white suspension was filtered through a mixture of Celite and SiO<sub>2</sub> with 1:1 CH<sub>2</sub>Cl<sub>2</sub>-Et<sub>2</sub>O and concentrated to give **106** (0.53 g, 100%) as an oil.



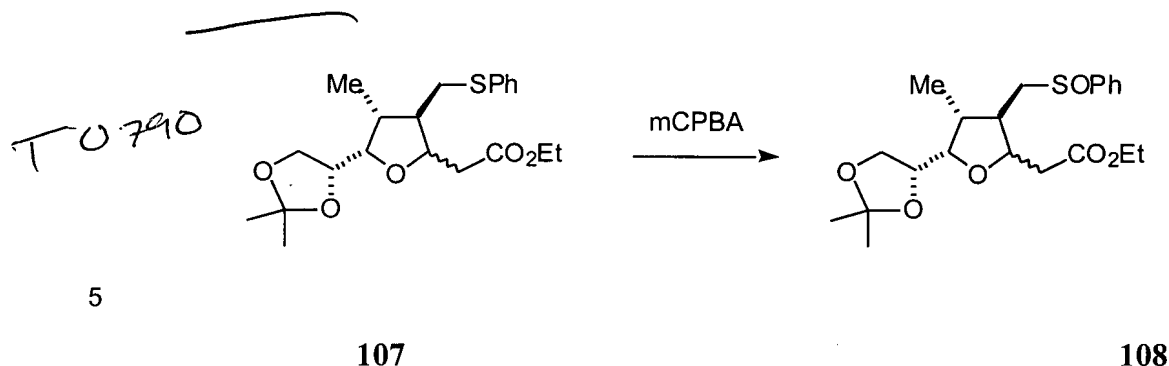
15

106

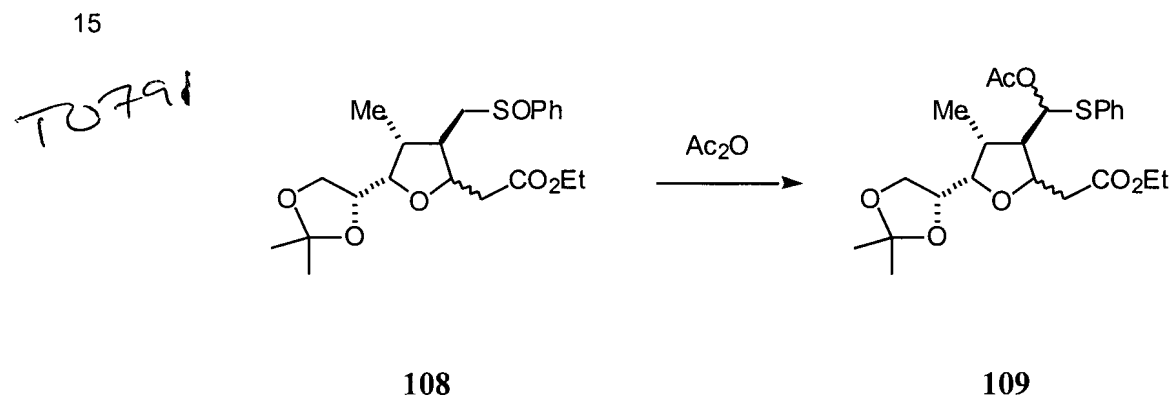
107

**Compound 107** A mixture of **106** (0.53 g, 1.64 mmol) and ethyl (triphenylphosphoranylidene)acetate (1.15 g, 3.29 mmol) in toluene (10 mL) was heated to 80 °C for 15 h. The mixture was cooled to rt and DBU (25 μL, 0.16 mmol) was introduced. The mixture was heated to 80 °C for 1.5 h, cooled to rt, concentrated and purified by column

chromatography (10% to 20% EtOAc-hexanes) to give **107** (0.54 g, 83%) as an oil (3:1 ratio of  $\alpha$ : $\beta$  isomers).

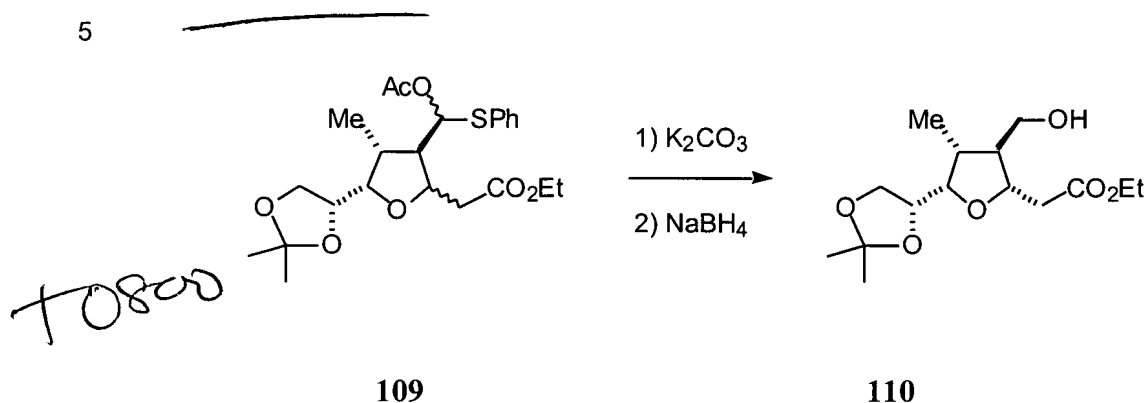


**Compound 108** A solution of mCPBA (~55%, 450 mg in 4.5 mL  $\text{CH}_2\text{Cl}_2$ , 1.44 mmol) was added to a solution of **107** (0.54 g, 1.36 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) at  $-78^\circ\text{C}$ . The reaction mixture was diluted with saturated aqueous  $\text{NaHCO}_3$  (50 mL),  $\text{H}_2\text{O}$  (10 mL), and  $\text{Et}_2\text{O}$  (60 mL) and then warmed to rt. The separated aqueous layer was extracted with EtOAc (4 $\times$ ) and the combined organic phases were dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by column chromatography (50% EtOAc-hexanes) to give **108** (0.51 g, 92%) as an oil.



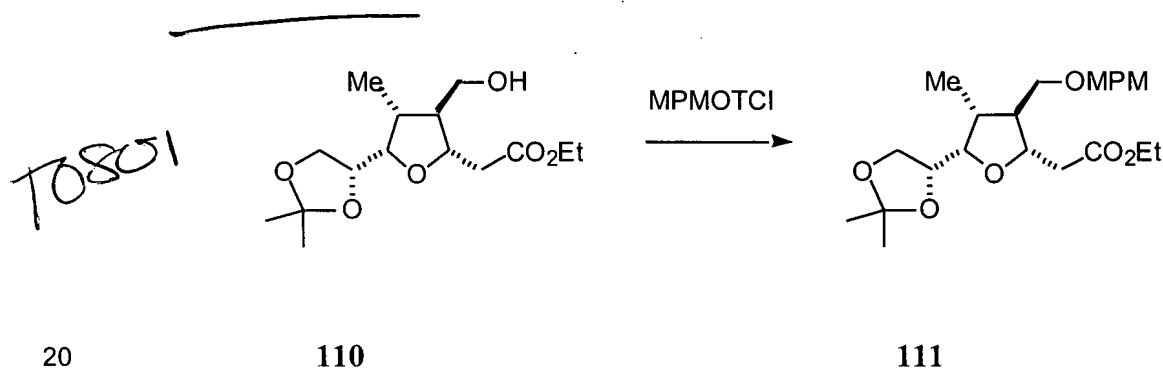
**Compound 109** A mixture of **108** (0.51 g, 1.24 mmol) and NaOAc (1.00 g, 12.4 mmol) in  $\text{Ac}_2\text{O}$  (10 mL) was stirred at  $140^\circ\text{C}$  for 12 h, cooled to rt and then concentrated. The residue was partitioned between saturated aqueous  $\text{NaHCO}_3$  (20 mL) and  $\text{Et}_2\text{O}$  (30 mL), and stirred

vigorously at rt for 30 min. The separated aqueous layer was extracted with Et<sub>2</sub>O (2×), and the combined organic phases were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (5% to 15% EtOAc-hexanes) to give **109** (0.41 g, 73%) as an oil.

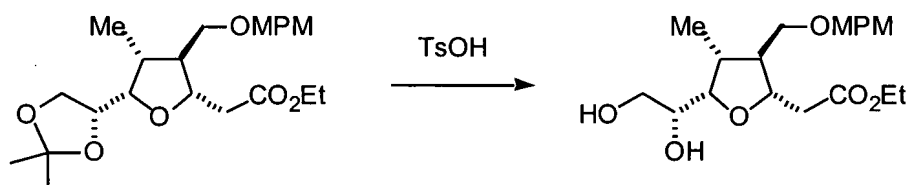


10 **Compound 110** A mixture of **109** (0.41 g, 0.91 mmol) and K<sub>2</sub>CO<sub>3</sub> (44.3 mg, 0.32 mmol) in EtOH (5 mL) was heated to 60–70 °C for 1 d. After cooling to rt, the reaction mixture was concentrated and eluted through a SiO<sub>2</sub> column (10% to 20% EtOAc-hexanes) to give the partially purified aldehyde intermediate. This material was dissolved in EtOH (2.5 mL), treated with NaBH<sub>4</sub> (50 mg, 1.32 mmol) and stirred at rt for 30 min. The mixture was concentrated and

15 purified by column chromatography (40% EtOAc-hexanes) to give **110** (181 mg, 66%).



**Compound 111**  $\text{BF}_3 \cdot \text{OEt}_2$  (0.05 M in  $\text{CH}_2\text{Cl}_2$ , 175  $\mu\text{L}$ , 8.75  $\mu\text{mol}$ ) was added to a solution of **110** (181 mg, 0.60 mmol) and *p*-methoxybenzyl 2,2,2-trichloroacetimidate (0.50 mL, 1.80 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 mL) at 0 °C. The resulting mixture was stirred for 1.5 h at 0 °C and for 2 h at rt until the reaction was complete. The mixture was quenched with saturated aqueous  $\text{NaHCO}_3$  (25 mL) and extracted with  $\text{Et}_2\text{O}$  (5 $\times$ ). The combined organic phases were dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by column chromatography ( $\text{CH}_2\text{Cl}_2$  and then 20% EtOAc-hexanes) to give semi-pure **111** (0.37 g, >100%) as an oil.



10

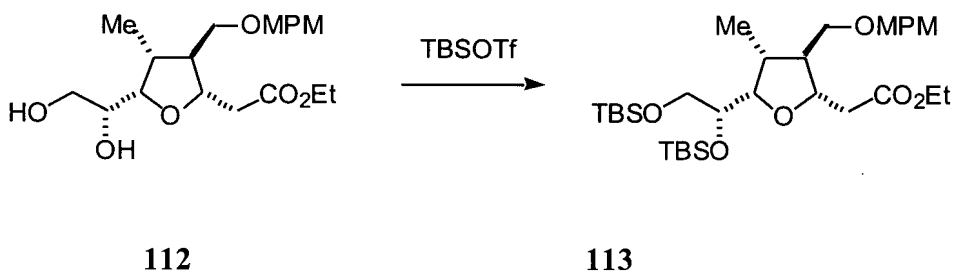
**111**

**112**

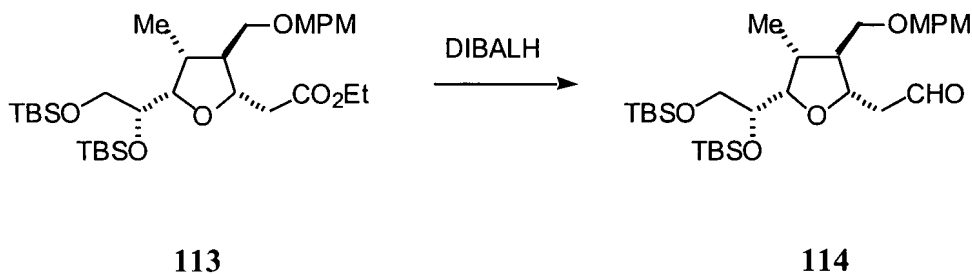
**Compound 112** A mixture of **111** (0.37 g, max. = 0.60 mmol) and  $\text{TsOH} \cdot \text{H}_2\text{O}$  (36 mg) in EtOH (5 mL) was stirred initially at rt overnight and then at 60 °C for 1 h. Additional  $\text{TsOH} \cdot \text{H}_2\text{O}$  (31 mg) was added at rt and the reaction mixture was stirred for 1 h at rt. The mixture was then concentrated, quenched with saturated aqueous  $\text{NaHCO}_3$  and extracted with EtOAc (5 $\times$ ). The combined organic phases were dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by column chromatography (20% to 50% EtOAc-hexanes and then 5% MeOH- $\text{CH}_2\text{Cl}_2$ ) to give **112** (121 mg, 53%) as an oil along with recovered **111** (49 mg, 21%).

20

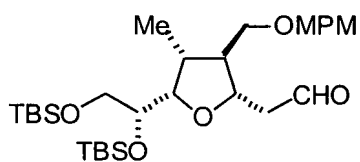




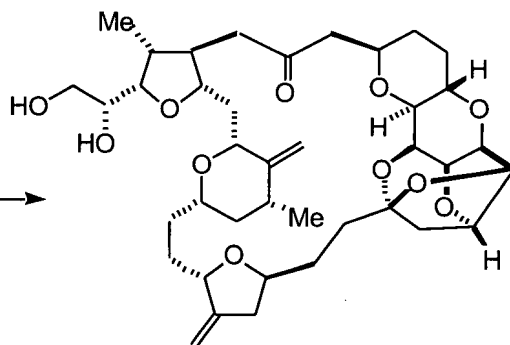
**Compound 113** TBSOTf (250  $\mu\text{L}$ , 1.09 mmol) was added to a solution of **112** (121 mg, 0.32 mmol) and  $\text{Et}_3\text{N}$  (176  $\mu\text{L}$ , 1.26 mmol) in  $\text{CH}_2\text{Cl}_2$  at 0  $^\circ\text{C}$  and the resulting mixture was stirred for 25 min. The reaction was quenched with saturated aqueous  $\text{NaHCO}_3$  (15 mL) and the separated aqueous layer was extracted with ether (3 $\times$ ). The combined organic phases were dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by column chromatography (5% to 10% EtOAc/hexanes) to give **113** (165 mg, 85%) as an oil.



**Compound 114** DIBALH (1 M in toluene, 0.54 mL, 0.54 mmol) was added to a solution of **113** (165 mg, 0.27 mmol) in toluene (5 mL) at -78  $^\circ\text{C}$  and the resulting mixture was stirred at -78  $^\circ\text{C}$  for 10 min. The reaction was quenched by careful addition of MeOH (65  $\mu\text{L}$ , 0.81 mmol) and  $\text{H}_2\text{O}$  (29  $\mu\text{L}$ , 0.81 mmol), warmed to rt and stirred for 25 min. The white suspension was filtered through Celite with 1:1  $\text{CH}_2\text{Cl}_2$ - $\text{Et}_2\text{O}$ . Concentration and purification by column chromatography (10% to 20% EtOAc-hexanes) gave **114** (153 mg, 100%) as an oil.

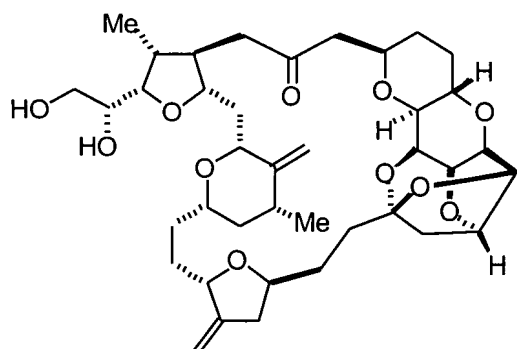


114

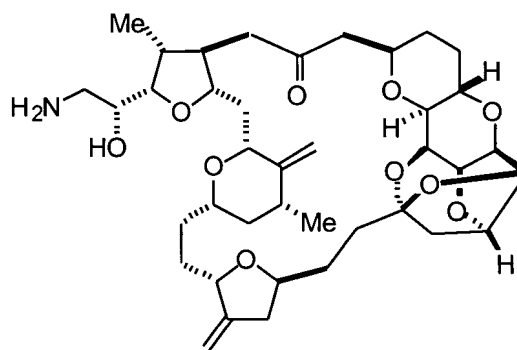


B2090

5 **B2090** In a manner similar to that described in Scheme 6 for the synthesis of **B1794**, intermediate **114** was converted to **B2090**. HRMS (FAB): calcd for  $C_{39}H_{56}O_{11}+Na$  723.3720. Found: 723.3731.



B2090

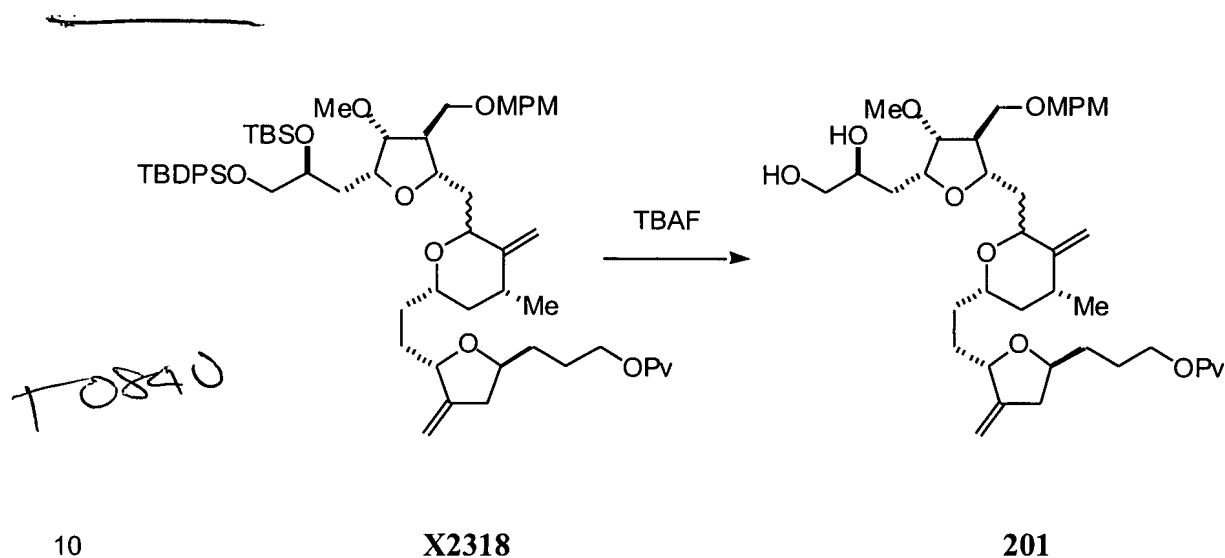


B2136

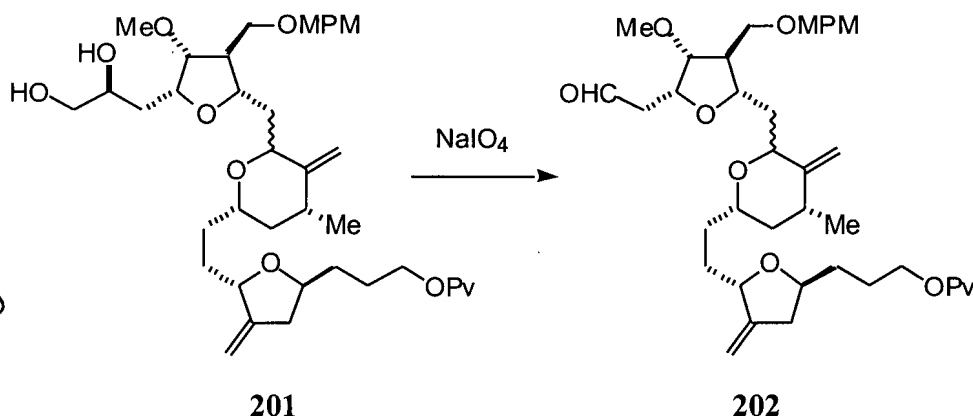
10

**B2136** In a manner analogous to that of **B1939**, **B2090** was converted to **B2136**. HRMS (FAB): calcd for  $C_{39}H_{57}NO_{10} + Na$  722.3880. Found: 722.3907.

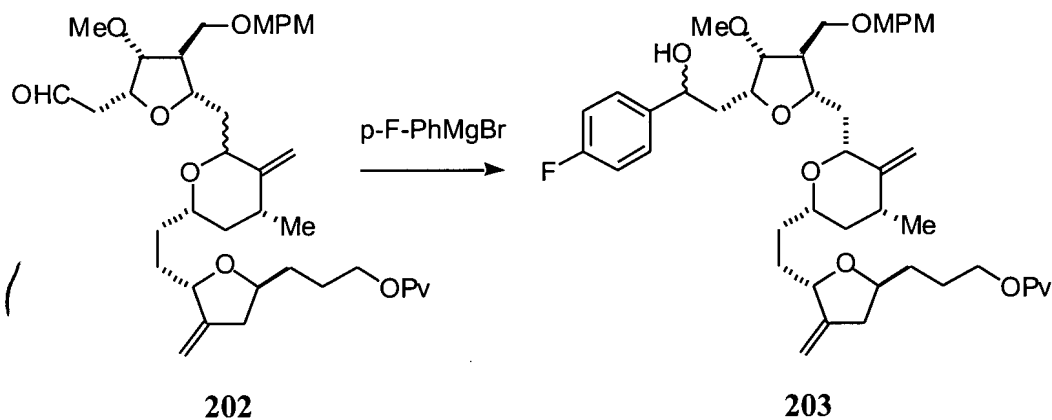
## 5 Synthesis of B2039/B2043:



**Diol 201** TBAF (1 M in THF, 383  $\mu$ L, 0.383 mmol) was added to a solution of **X2318** (**350-LS-218**) (80.8 mg, 0.0765 mmol) in THF (7 mL) and stirred at rt for 16 h. After partial concentration, the residue was loaded directly onto a  $SiO_2$  column packed using 30% EtOAc-hexanes. Gradient elution (30% EtOAc-hexanes to EtOAc) furnished diol **201** (49.7 mg, 92%).

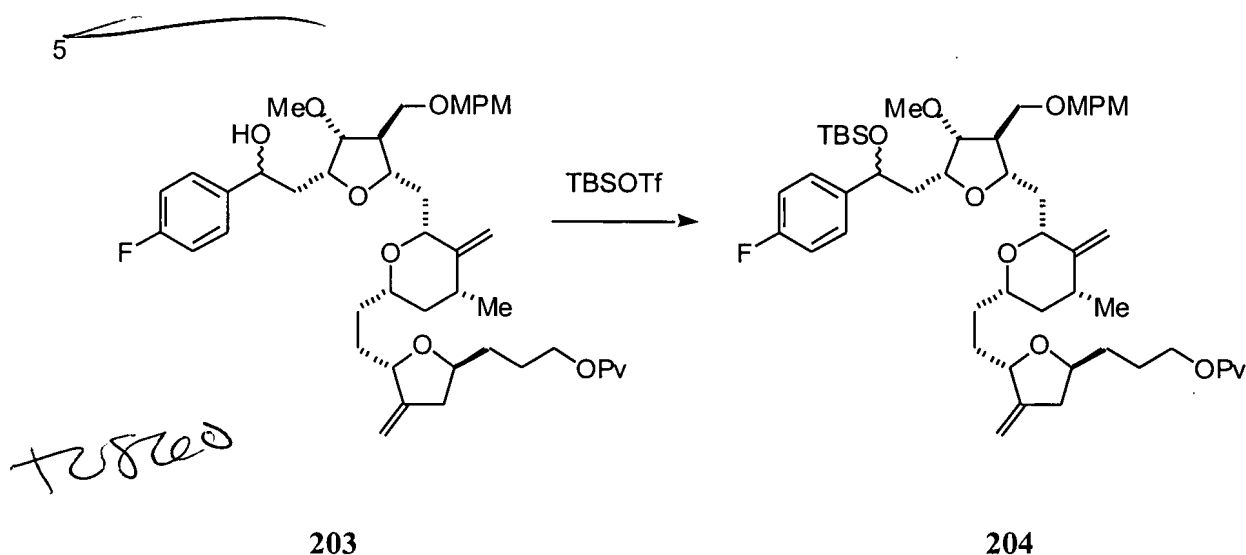


**Aldehyde 202** A mixture of diol **201** (49.7 mg, 0.0707 mmol), NaIO<sub>4</sub> (100 mg, 0.47 mmol), MeOH (10 mL) and H<sub>2</sub>O (2.5 mL) was stirred at rt for 30 min. H<sub>2</sub>O was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (4×). The combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (30% EtOAc-hexanes) to provide aldehyde **202** (41.7 mg, 88%).



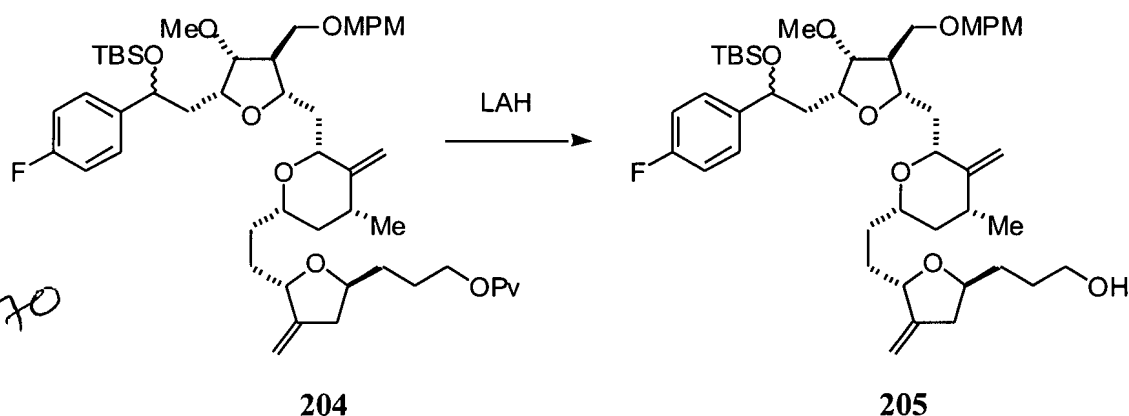
**Alcohol 203** 4-Fluorophenylmagnesium bromide (2 M in Et<sub>2</sub>O, 155 μL, 0.31 mmol) was added to a solution of aldehyde **202** (41.7 mg, 0.062 mmol) in THF (6 mL). After 15 min at rt, the reaction was quenched with saturated aqueous NH<sub>4</sub>Cl and extracted with CH<sub>2</sub>Cl<sub>2</sub> (4×). The combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by preparative

TLC (40% EtOAc-hexanes) to provide alcohol **203** (32.4 mg, 68%) as a 1:1 mixture of C34 isomers. The minor undesired C27 isomer was separated at this stage and was also isolated as a 1:1 mixture of C34 isomers (8.4 mg, 18%).



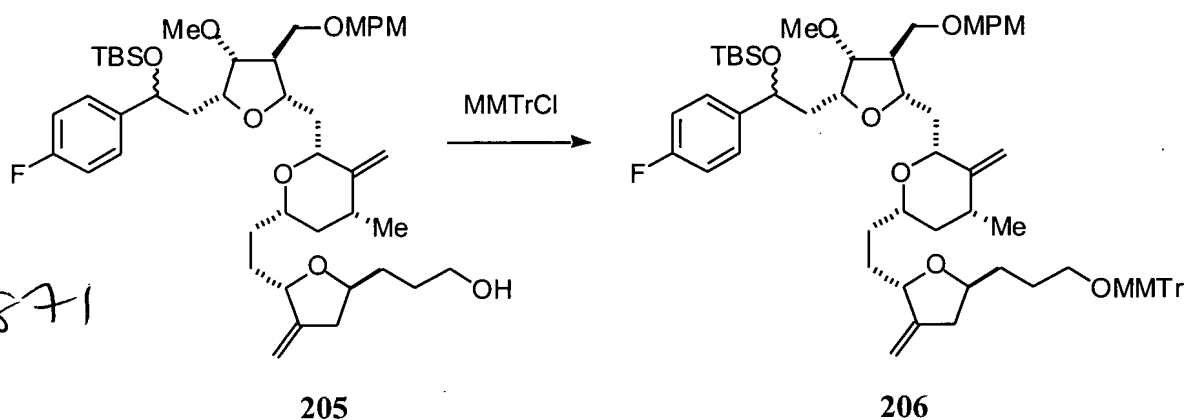
10 **Ether 204** Et<sub>3</sub>N (18  $\mu$ L, 0.13 mmol) and TBSOTf (15  $\mu$ L, 0.063 mmol) were added to a solution of alcohol **203** (32.4 mg, 0.042 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) at 0 °C. After 20 min the reaction was quenched by the addition of saturated aqueous NH<sub>4</sub>Cl and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 $\times$ ). The combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide ether **204** (33.1 mg, 89%).

15



5 **Alcohol 205** LAH (1 M in THF, 113  $\mu\text{L}$ , 0.113 mmol) was added dropwise to a solution of ether **204** (33.1 mg, 0.0375 mmol) in  $\text{Et}_2\text{O}$  (10 mL) at 0  $^\circ\text{C}$ . After 20 min,  $\text{H}_2\text{O}$  and 1 M NaOH were added and the mixture was stirred at rt for 10 min. Filtration through Celite, concentration and purification by column chromatography (40% EtOAc-hexanes) furnished alcohol **205** (28.4 mg, 95%).

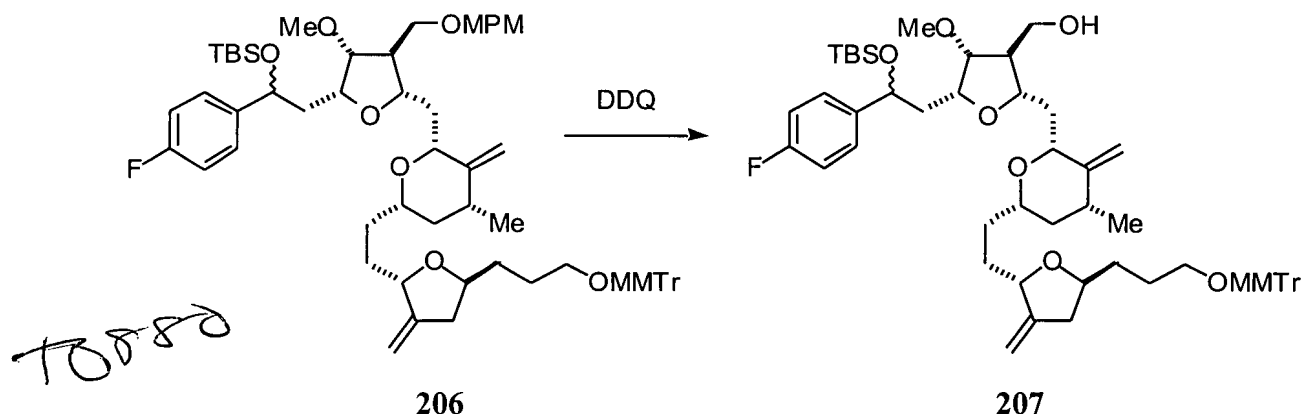
10



15 **Ether 206** Diisopropylethylamine (31  $\mu\text{L}$ , 0.18 mmol) and MMTrCl (22 mg, 0.071 mmol) were added to a solution of alcohol **205** (28.4 mg, 0.0356 mmol) in  $\text{CH}_2\text{Cl}_2$  (4 mL) at

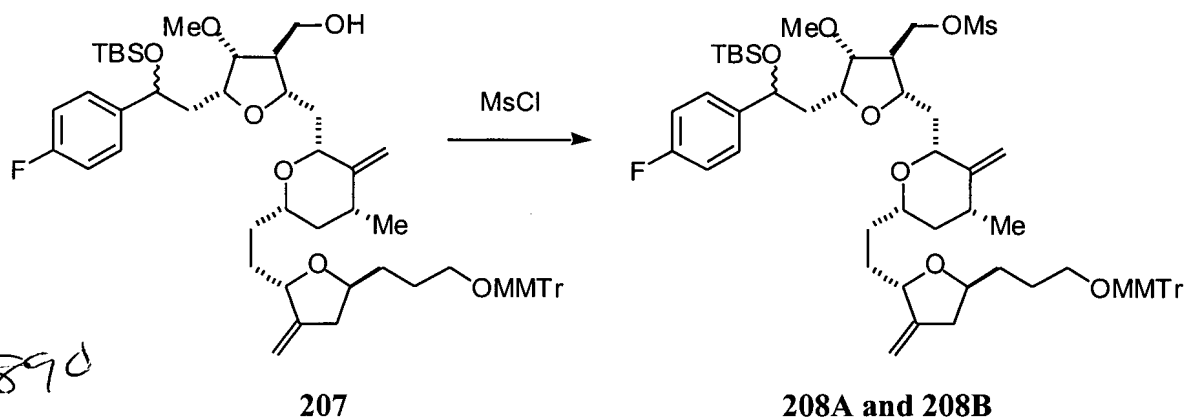
0 °C. After 15 h at rt, H<sub>2</sub>O was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×). The combined extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by preparative TLC (40% EtOAc-hexanes) to provide ether **206** as a ~1.5:1 mixture of C34 epimers (45 mg, quant), which contained a small amount of close-running impurities.

5



10 **Alcohol 207** DDQ (40 mg, 0.18 mmol) was added to a solution of ether **206** (37 mg, 0.034 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (4 mL) and a 1:10 mixture of *t*BuOH:pH 7 phosphate buffer (2 mL) at 0 °C. The mixture was stirred vigorously in the dark for 15 min. Three additional portions of DDQ (40 mg, 0.18 mmol) were added at 10 min intervals, then the reaction was diluted with saturated aqueous NaHCO<sub>3</sub> and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×). The combined organic extracts were

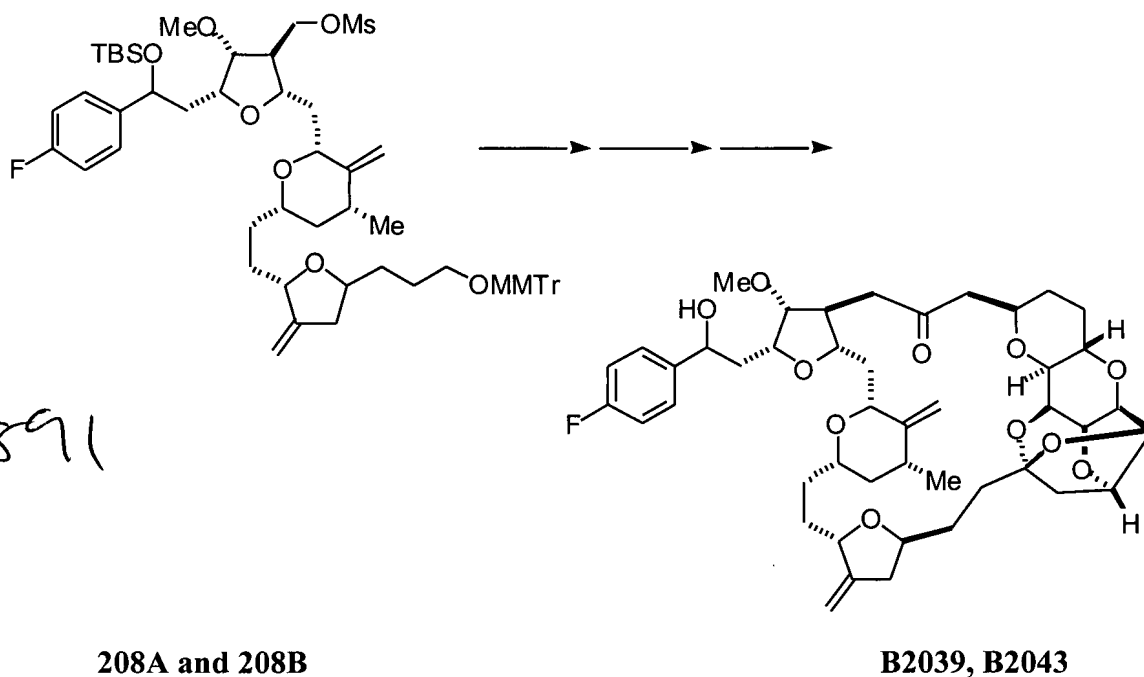
15 washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by preparative TLC (30% EtOAc-hexanes) to provide alcohol **207** (19.2 mg, 59%) as well as recovered ether **206** (9.7 mg, 26%).



**Mesylates 208A and 208B** Et<sub>3</sub>N (19  $\mu$ L, 0.13 mmol) and Ms<sub>2</sub>O (10 mg, 0.056 mmol)

5 were sequentially added to a solution of alcohol **207** (21.3 mg, 0.022 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) at 0 °C. After 30 min, saturated aqueous NaHCO<sub>3</sub> was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 $\times$ ). The combined extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by preparative TLC (30% EtOAc-hexanes) to provide mesylates **208A** (11.7 mg, 51%) and **208B** (6.5 mg, 28%) as single C34 isomers.

10



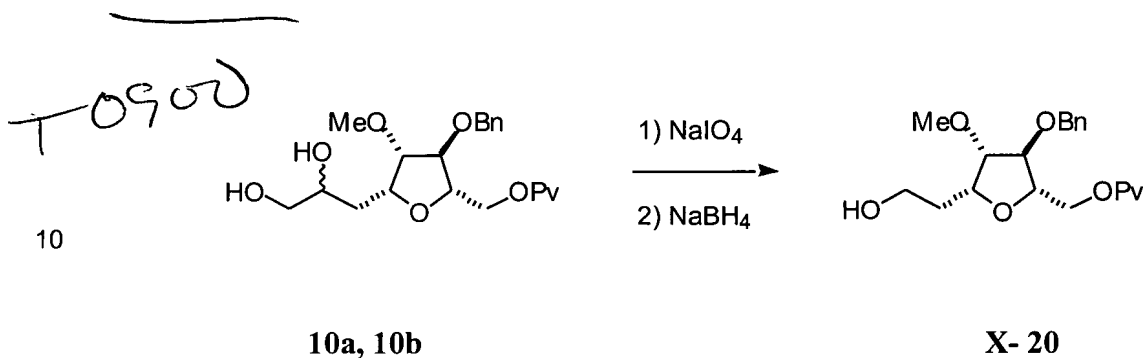


**B2039** and **B2043** In a manner similar to that described in Scheme 6 for the synthesis of **B1794**, both diastereomers **208A** and **208B** were independently converted to **B2039** and **B2043**.

HRMS (FAB): calcd for  $C_{45}H_{59}FO_{11} + Na$  817.3939. Found: for **B2039** 817.3896, **B2043**

5 817.3910.

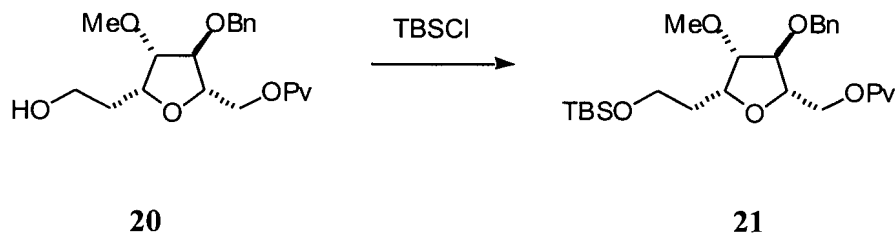
### Synthesis of **B2086**, **B2088**, **B2091**



**Alcohol X-20**  $NaIO_4$  (1.16 g, 5.4 mmol) was added to a solution of diols **10 a, b** (1.19 g, 3.0 mmol) in MeOH- $H_2O$  (4:1, 75 mL) at 0 °C. The reaction mixture was allowed to warm to rt. After stirring for 40 min, the mixture was diluted with EtOAc, filtered through Celite, concentrated, and partitioned between brine and  $CH_2Cl_2$ . The separated aqueous layer was extracted with  $CH_2Cl_2$  (2×). The combined organic layers were dried over  $Na_2SO_4$  and concentrated to furnish the crude aldehyde intermediate.

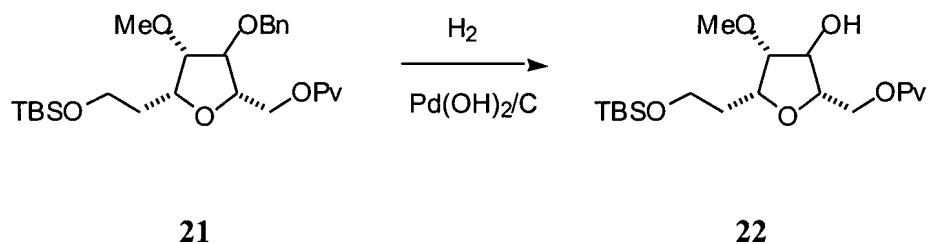
$NaBH_4$  (228 mg, 6.0 mmol) was added to a solution of the aldehyde in MeOH- $Et_2O$  (1:1, 40 mL) at 0 °C. The mixture was stirred for 30 min, carefully quenched with saturated aqueous  $NH_4Cl$ , stirred for 20 min at rt and extracted with  $CH_2Cl_2$  (3×). The combined extracts were dried over  $Na_2SO_4$ , concentrated and purified by flash chromatography (40% to 50% EtOAc-hexanes) to afford alcohol **X-20** (1.02 g, 93% for two steps).

25



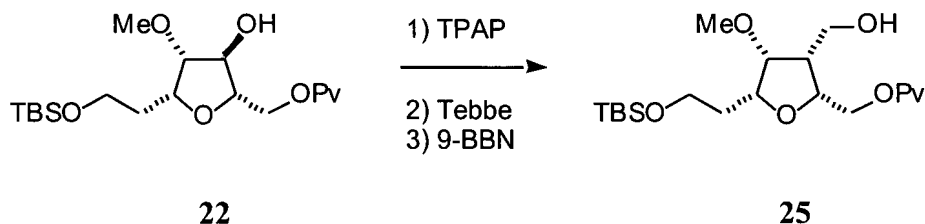
5 **Silyl ether 21** Imidazole (0.94 g, 13.9 mmol) and TBSCl (0.59 g, 3.89 mmol) were added sequentially to a solution of alcohol **X-20** (1.02 g, 2.78 mmol) in DMF (10 mL) at rt. After 14 h, the reaction mixture was diluted with saturated aqueous  $\text{NH}_4\text{Cl}$  and extracted with EtOAc (3 $\times$ ). The combined organic extracts were washed with  $\text{H}_2\text{O}$ , brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by flash chromatography (5% to 15% EtOAc-hexanes) to afford silyl ether **21** (1.3 g, 98%).

10



15 **Alcohol 22** A mixture of  $\text{Pd(OH)}_2$  (20%, 0.8 g), silyl ether **21** (1.3 g, 2.70 mmol) and EtOAc (30 mL) was stirred for 1 h under 1 atm  $\text{H}_2$  at rt, filtered through Celite, concentrated and purified by flash chromatography (20% to 40% EtOAc-hexanes) to afford alcohol **22** (0.96 g, 91%).

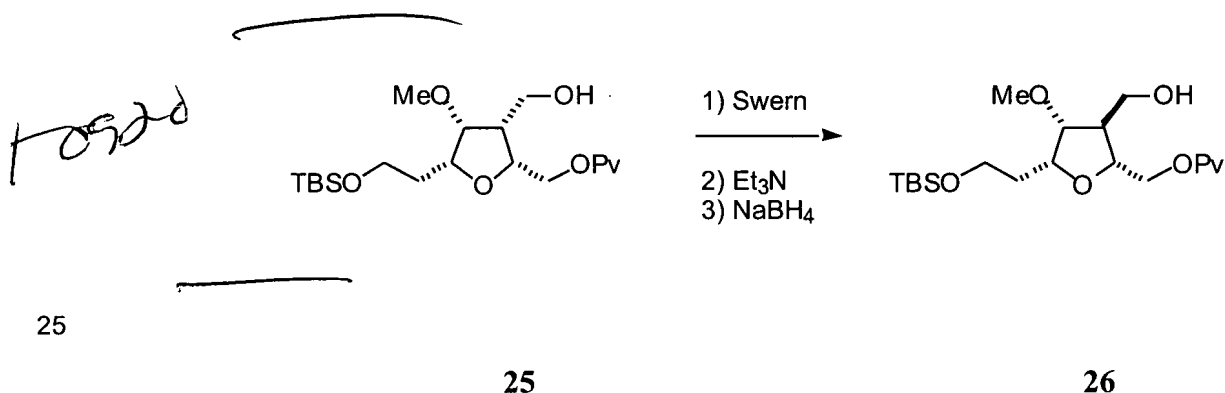
20



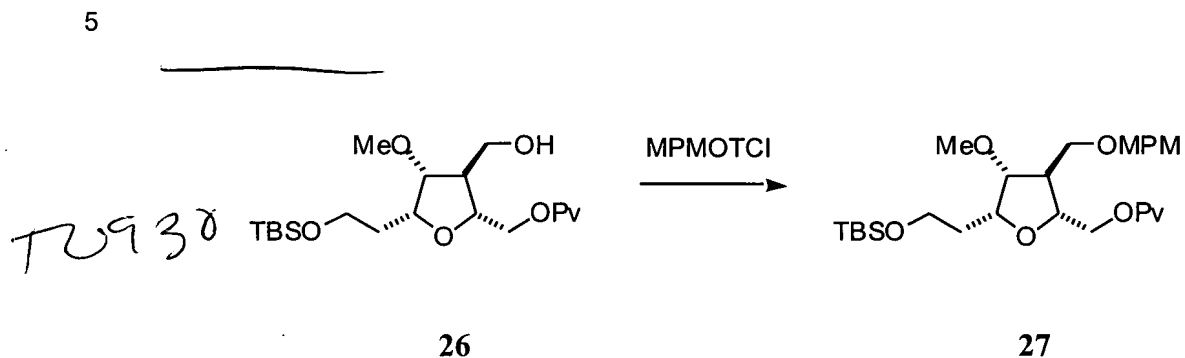
**Alcohol 25** 4-Methylmorpholine N-oxide (980 mg, 8.4 mmol) and TPAP (131 mg, 3.26 mmol) were added sequentially to a solution of alcohol **22** (1.78 g, 4.6 mmol) in  $\text{CH}_2\text{Cl}_2$  (45 mL) at rt. A cold bath was necessary to control the exotherm. After 20 min, the reaction mixture was  
5 diluted with hexanes, filtered through a short  $\text{SiO}_2$  column (15% EtOAc-hexanes) and concentrated to give the crude ketone.

Tebbe reagent (14.9 mL, 9.0 mmol) was added over 10 min to a solution of the crude ketone in THF (60 mL) at 0 °C. After 20 min, the reaction mixture was poured into  $\text{Et}_2\text{O}$  (100 mL) that was precooled to -78 °C, quenched by slow addition of  $\text{H}_2\text{O}$  (30 mL), warmed to rt.,  
10 stirred for 30 min and extracted with  $\text{Et}_2\text{O}$  (4×). The combined extracts were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by flash chromatography (10% EtOAc-hexanes) to afford the desired olefin contaminated by the gem-dimethyl product (1.07 g). This mixture was used directly in the next step.

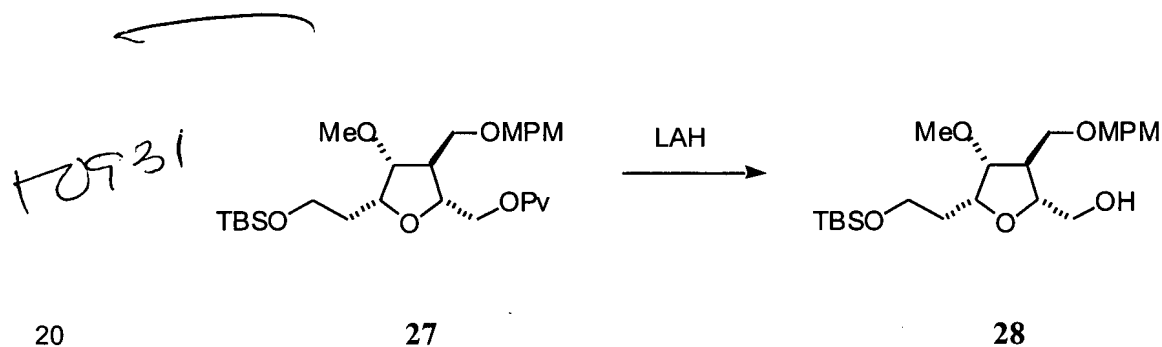
9-BBN (0.5 M in THF, 11.6 mL, 5.8 mmol) was added to a solution of the olefin in THF  
15 (15 mL) at 0 °C. The reaction mixture was allowed to warm to rt, stirred for 5 h and then recooled to 0 °C.  $\text{H}_2\text{O}$  (60 mL), THF (60 mL) and  $\text{NaBO}_3 \cdot 4 \text{H}_2\text{O}$  (5.7 g) were added. After stirring for 5 h at rt, the THF was removed under reduced pressure and the aqueous residue was extracted with EtOAc (4×). The combined organic extracts were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by flash chromatography (20% to 40% EtOAc-hexanes) to  
20 furnish alcohol **25** (605 mg, 18% for three steps).



**Alcohol 26** Using the procedure previously described, alcohol **25** (604 mg, 1.49 mmol) was sequentially oxidized, isomerized, and reduced. Purification by flash chromatography (20% to 40% EtOAc-hexanes) afforded alcohol **26** (550 mg, 91% for three steps).

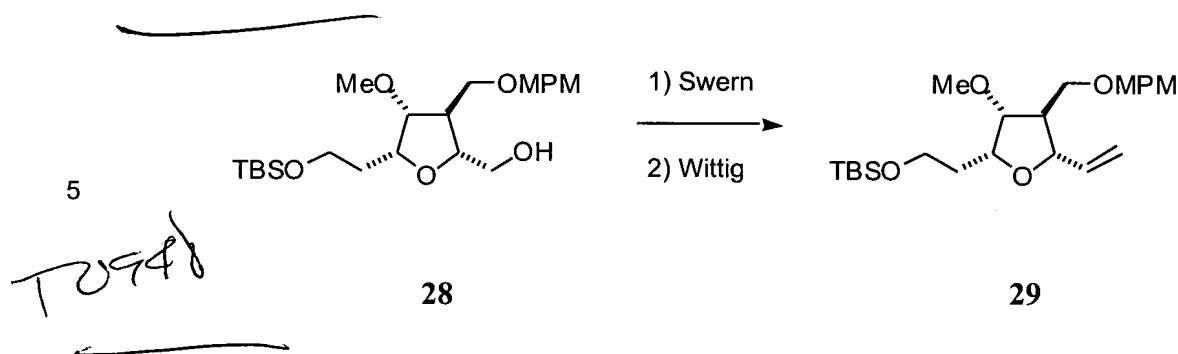


**MPM-ether 27**  $\text{BF}_3 \cdot \text{OEt}_2$  (0.05 M in  $\text{CH}_2\text{Cl}_2$ , 270  $\mu\text{L}$ , 0.013 mmol) was added to a solution of alcohol **26** (545 mg, 1.35 mmol) and MPM-trichloroimidate (1.14 g, 4.0 mmol) in  $\text{CH}_2\text{Cl}_2$  (40 mL) at 0 °C. After 1 h, the reaction was quenched with saturated aqueous  $\text{NaHCO}_3$ , extracted with  $\text{CH}_2\text{Cl}_2$ , dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by flash chromatography (10% to 15% EtOAc-hexanes) to afford MPM-ether **27** (580 mg, 82%).



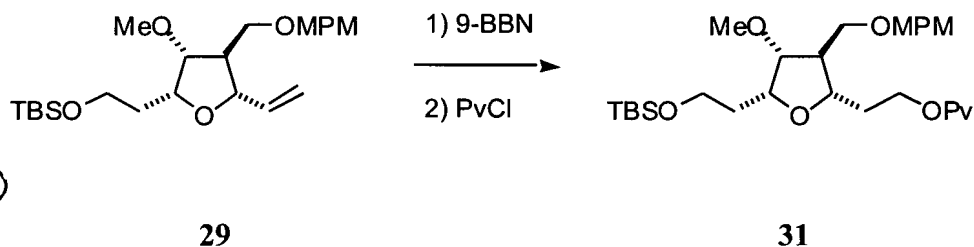
**Alcohol 28** LAH (1 M in THF, 1.9 mL, 1.9 mmol) was added to a solution of MPM-ether **27** (580 mg, 1.11 mmol) in  $\text{Et}_2\text{O}$  (100 mL) at 0 °C. After 30 min, the reaction was quenched carefully with  $\text{H}_2\text{O}$  (0.5 mL), and 1 N aqueous  $\text{NaOH}$  (0.5 mL), stirred for 1 h at rt,

filtered through Celite, concentrated and purified by flash chromatography (30% to 50% EtOAc-hexanes) to afford alcohol **28** (460 mg, 95%).



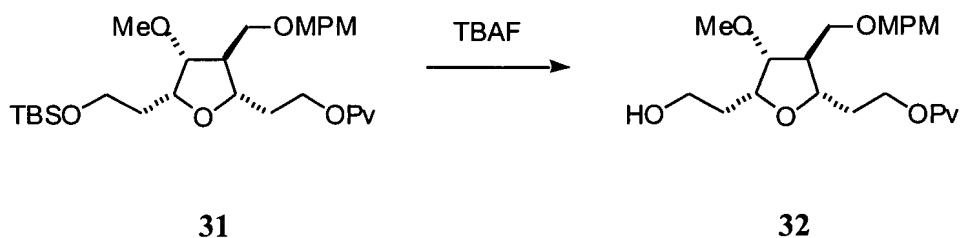
**Olefin 29** DMSO (441  $\mu$ L, 6.23 mmol) was added to a solution of oxalyl chloride (272  $\mu$ L, 3.12 mmol) in  $\text{CH}_2\text{Cl}_2$  (30 mL) at  $-78^\circ\text{C}$ . After 15 min, a solution of alcohol **28** (458 mg, 1.04 mmol) in  $\text{CH}_2\text{Cl}_2$  (15 mL) was added to the reaction mixture. After stirring for 1 h at  $-78^\circ\text{C}$ ,  $\text{Et}_3\text{N}$  (1.3 mL, 9.35 mmol) was added. The reaction mixture was warmed to  $0^\circ\text{C}$ , stirred for 10 min, diluted with saturated aqueous  $\text{NH}_4\text{Cl}$  and extracted with  $\text{CH}_2\text{Cl}_2$  (3 $\times$ ). The combined organic extracts were dried over  $\text{Na}_2\text{SO}_4$ , concentrated and filtered through a short  $\text{SiO}_2$  column (20% to 30% EtOAc-hexanes) to provide the crude aldehyde.

$n\text{-BuLi}$  (1.63 M, 1.4 mL, 2.28 mmol) was added dropwise to a solution of  $\text{CH}_3\text{PPh}_3\text{Br}$  (815 mg, 2.28 mmol), THF (20 mL) and DMSO (7.5 mL) at  $0^\circ\text{C}$ . After 1 h, a solution of the aldehyde in THF (10 mL) was added. The reaction mixture was warmed to rt and stirred for 3 h. Saturated aqueous  $\text{NH}_4\text{Cl}$  was added and the mixture was extracted with EtOAc (4 $\times$ ). The combined organic extracts were washed with  $\text{H}_2\text{O}$ , brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by flash chromatography (10% to 15% EtOAc-hexanes) to afford olefin **29** (380 mg, 95% yield for 2 steps).



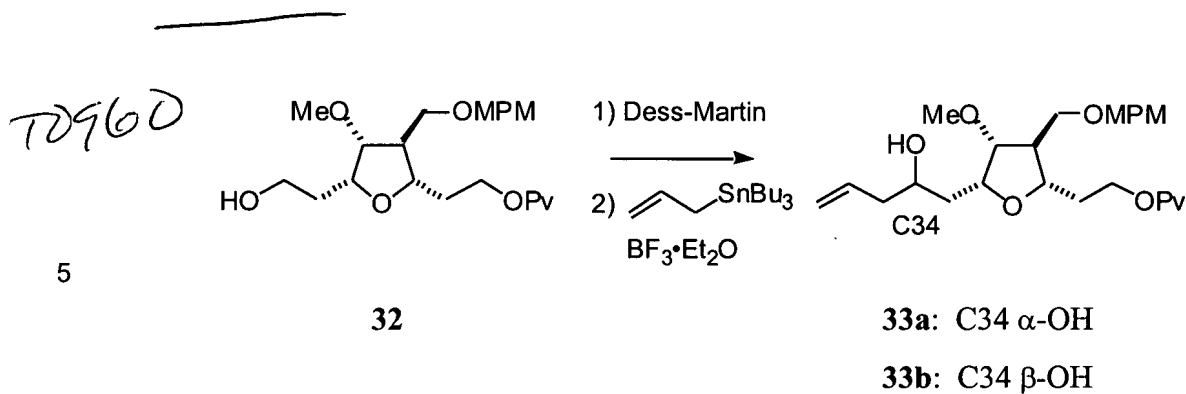
5 **Compound 31** 9-BBN (0.5 M in THF, 6 mL, 3 mmol) was added to a solution of olefin **29** (370 mg, 0.85 mmol) in THF (7 mL) at 0 °C. The mixture was allowed to warm to rt and stirred for 1 h. After recooling to 0 °C, H<sub>2</sub>O (30 mL), THF (20 mL), and NaBO<sub>3</sub>•4 H<sub>2</sub>O (2.8 g) were added. After stirring for 3 h at rt, the THF was removed under reduced pressure. The aqueous residue was extracted with EtOAc (4×), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by  
10 flash chromatography (25% to 50% EtOAc-hexanes) to afford alcohol **30** which was used directly in the next step.

Pivaloyl chloride (157 μL, 1.27 mmol) was added to a solution of alcohol **30** in CH<sub>2</sub>Cl<sub>2</sub>-pyridine (1:1 mixture, 10 mL) at rt. After 18 h, additional pivaloyl chloride (100 μL, 0.81 mmol) was added. After 1 h, the reaction mixture was cooled to 0 °C, quenched with MeOH (0.5 mL),  
15 concentrated, diluted with brine and extracted with CH<sub>2</sub>Cl<sub>2</sub> (4×). The combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by flash chromatography (10% to 15% EtOAc-hexanes) to afford compound **31** (410 mg, 90% for two steps).



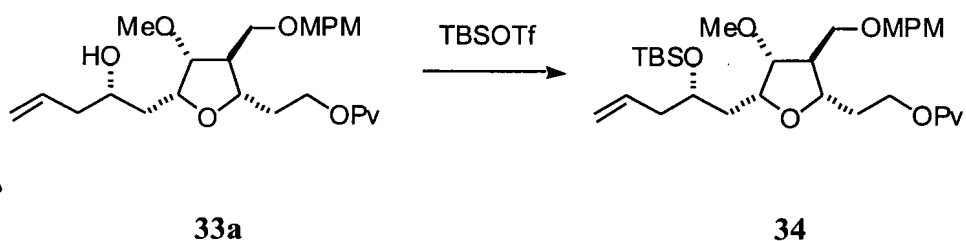
**Alcohol 32** TBAF (1 M in THF, 1.14 mL, 1.14 mmol) was added to a solution of **31**  
25 (410 mg, 0.761 mmol) in THF (5 mL) at rt. After 1.5 h, the reaction mixture was concentrated

and purified by flash chromatography (40% EtOAc-hexanes to 100% EtOAc) to afford alcohol **32** (320 mg, 100%).

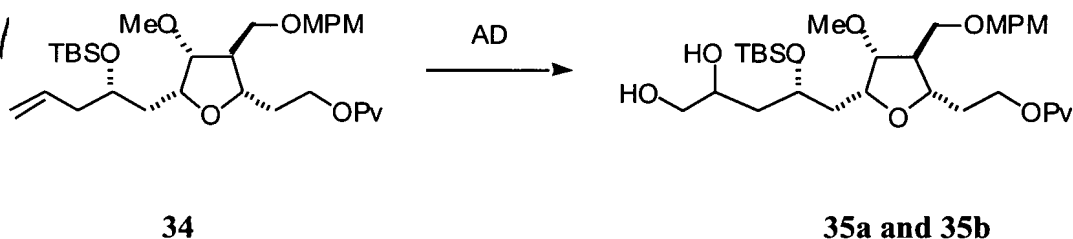


**Alcohols 33a and 33b** Dess-Martin periodinane (925 mg, 2.18 mmol) was added to a solution of alcohol **32** (309 mg, 0.727 mmol) in  $\text{CH}_2\text{Cl}_2$  (19 mL) at rt. After 1 h, the reaction was diluted with  $\text{Et}_2\text{O}$  and filtered through Celite. The filtrate was washed sequentially with a 1:9 mixture of saturated aqueous  $\text{NaHCO}_3$ - $\text{Na}_2\text{S}_2\text{O}_3$  and brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by flash chromatography (20% to 30% EtOAc-hexanes) to afford the desired aldehyde, which was taken immediately through the next step.

**BF<sub>3</sub>•OEt<sub>2</sub>** (135  $\mu\text{L}$ , 1.1 mmol) was added to a solution of the crude aldehyde, tri-*n*-butylallyltin (337  $\mu\text{L}$ , 1.08 mmol) and  $\text{CH}_2\text{Cl}_2$  (16 mL) at  $-78^\circ\text{C}$ . After 1 h, the reaction was quenched with saturated aqueous  $\text{NaHCO}_3$  and extracted with  $\text{CH}_2\text{Cl}_2$  (3 $\times$ ). The combined organic extracts were dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by MPLC (25% to 30% EtOAc-hexanes) to afford the major, more polar alcohol **33a** (165 mg, 49% for two steps) and the minor less polar product **33b** (90 mg, 27% for two steps).

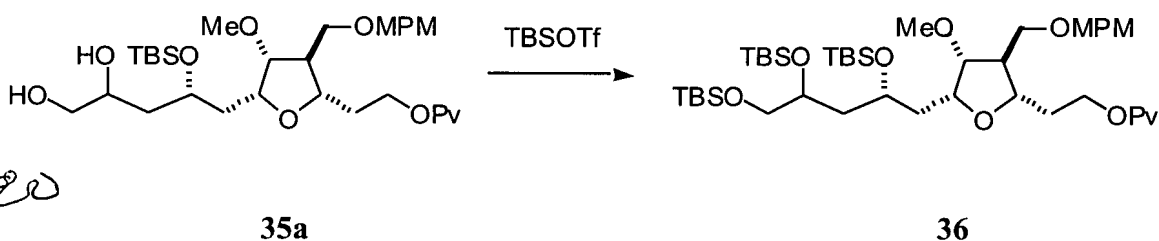


**Compound 34** TBSOTf (163  $\mu\text{L}$ , 0.710 mmol) was added to a solution of alcohol **33a** (165 mg, 0.355 mmol),  $\text{Et}_3\text{N}$  (247  $\mu\text{L}$ , 1.78 mmol) and  $\text{CH}_2\text{Cl}_2$  (5 mL) at 0  $^\circ\text{C}$ . After 25 min, the reaction was quenched with saturated aqueous  $\text{NaHCO}_3$ , extracted with  $\text{CH}_2\text{Cl}_2$  (3 $\times$ ), dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by flash chromatography (15% to 20% EtOAc-hexanes) to afford compound **34** (200 mg, 98%).



**Diols 35a and 35b**  $\text{OsO}_4$  (0.1 M solution in toluene, 32  $\mu\text{L}$ , 3.2  $\mu\text{mol}$ ) was added to a solution of  $\text{K}_2\text{CO}_3$  (168 mg, 1.22 mmol),  $\text{K}_3\text{Fe}(\text{CN})_6$  (400 mg, 1.22 mmol),  $(\text{DHQ})_2\text{PYR}$  (11 mg, 12  $\mu\text{mol}$ ),  $\text{H}_2\text{O}$  (3.2 mL) and  $t\text{-BuOH}$  (2.2 mL) at 0  $^\circ\text{C}$ . Then a solution of olefin **34** (200 mg, 0.345 mmol) in  $t\text{-BuOH}$  (1 mL) was added to the reaction mixture. After 5 h at 0  $^\circ\text{C}$ ,  $\text{Na}_2\text{S}_2\text{O}_5 \cdot 5\text{H}_2\text{O}$  (200 mg) was added. The reaction mixture was warmed to rt, stirred for 30 min and extracted with  $\text{CH}_2\text{Cl}_2$  (5 $\times$ ). The combined organic extracts were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by preparative TLC (70% EtOAc- hexanes) to afford the major, less polar diol **35a** (118 mg, 56%), and minor, more polar diastereomeric product **35b** (74 mg, 35%). The individual diastereomers were each carried forward separately.

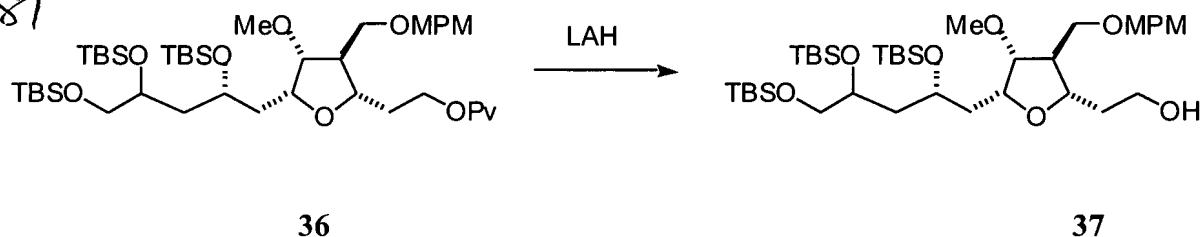




5 \_\_\_\_\_

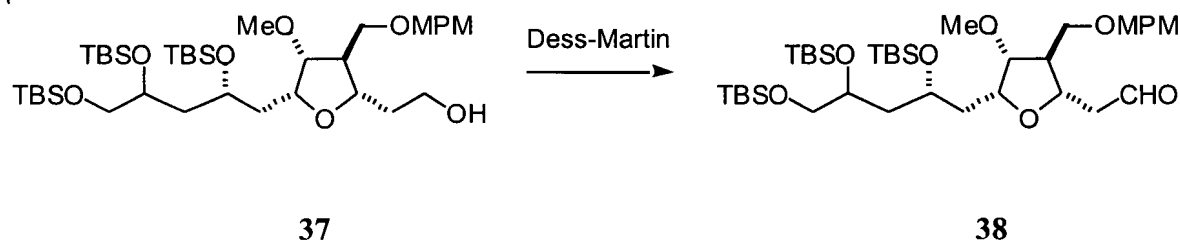
**Compound 36** TBSOTf (177  $\mu$ L, 0.77 mmol) was added to a solution of diol **35a** (118 mg, 0.192 mmol), Et<sub>3</sub>N (267  $\mu$ L, 1.92 mmol) and CH<sub>2</sub>Cl<sub>2</sub> (5 mL) at 0 °C. After 25 min, the reaction was quenched with saturated aqueous NaHCO<sub>3</sub>, extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 $\times$ ), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by flash chromatography (10% to 15% EtOAc-hexanes) to

10 afford compound **36** (161 mg, 100%).

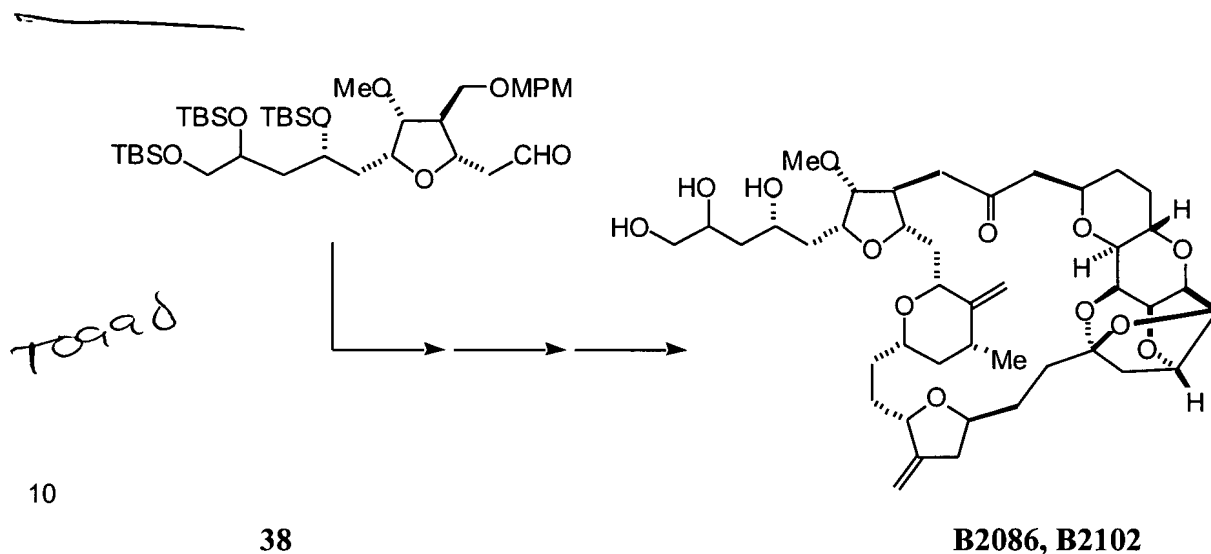


15 \_\_\_\_\_

**Alcohol 37** Using the procedure described previously for the preparation of alcohol **28**, compound **36** (161 mg, 0.192 mmol) afforded alcohol **37** (135 mg, 93%) after purification by flash chromatography (20% to 40% EtOAc-hexanes).

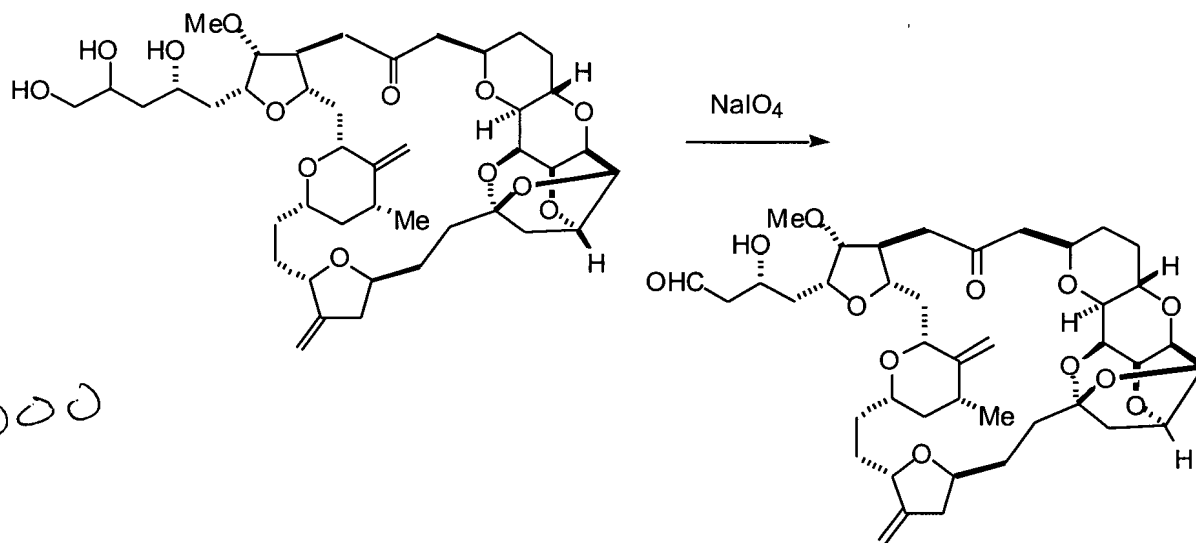


**Aldehyde 38** Dess-Martin periodinane (227 mg, 0.535 mmol) was added to a solution of alcohol **37** (135 mg, 0.178 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 mL) at rt. After 1 h, the reaction mixture was diluted with  $\text{Et}_2\text{O}$  and filtered through Celite. The filtrate was washed sequentially with a 1:9 mixture of saturated aqueous  $\text{NaHCO}_3$ - $\text{Na}_2\text{S}_2\text{O}_3$  and brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by flash chromatography (10% to 20% EtOAc-hexanes) to afford aldehyde **38** (127 mg, 95%).



**B2086, B2102** Each of the diastereomers obtained above were separately carried to final product in a manner similar to that described in scheme 6 for **B1794**. Diastereomer **35a** afforded **B2086**. Diastereomer **35b** afforded **B2102**.

7000

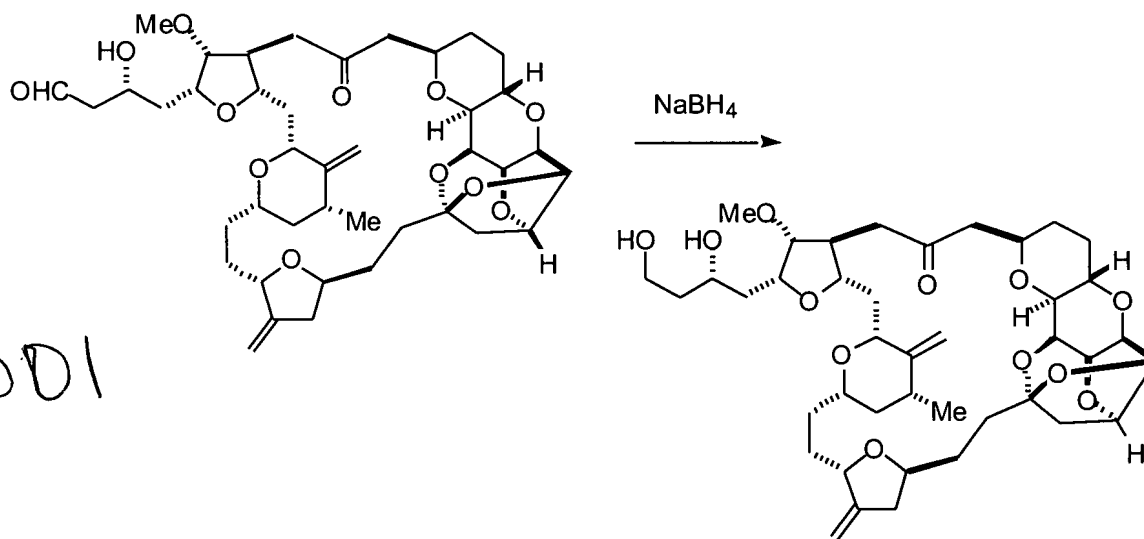


**B2086**

**B2088**

**B2088** NaIO<sub>4</sub> was added to a solution of **B2086** (1 mg, 1.29 μmol) in MeOH-H<sub>2</sub>O (4:1, 1 mL) at rt. After 30 min, the reaction mixture was diluted with H<sub>2</sub>O, extracted with CH<sub>2</sub>Cl<sub>2</sub> (6×), dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated to afford **B2088** (1.2 mg).

7001



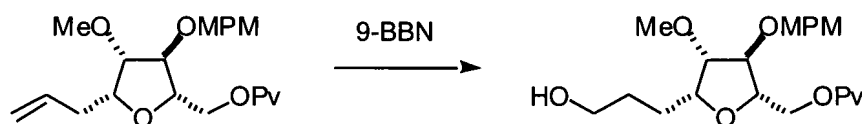
**B2088**

**B2091**

100

**B2091** NaBH<sub>4</sub> (0.013 M in EtOH, 20 μL, 0.27 μmol) was added to a solution of **B2088** (1 mg, 1.29 μmol) in MeOH-CH<sub>2</sub>Cl<sub>2</sub> (4:1, 0.5 mL) at -78 °C. Additional NaBH<sub>4</sub> was periodically added with close monitoring of the reaction by TLC (total of 220 μL of the NaBH<sub>4</sub> solution was required). The reaction mixture was quenched at 0 °C with saturated aqueous NH<sub>4</sub>Cl, stirred for 20 min at rt and extracted with CH<sub>2</sub>Cl<sub>2</sub> (6×). The combined extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by preparative TLC (7% MeOH-EtOAc) to furnish **B2091** (0.40 mg, 50%).

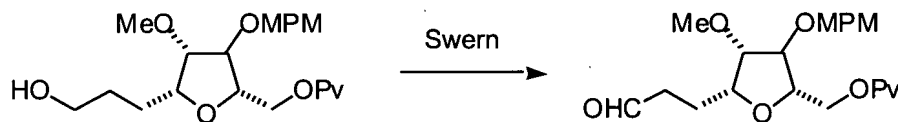
Synthesis of B1933:



**302**

**303**

**Alcohol 303** 9-BBN (0.5 M in THF, 23 mL, 0.012 mol) was added dropwise over 30 min to a solution of alkene **302** (1.51 g, 0.00386 mol) in THF (40 mL) at 0 °C. After stirring at rt for 80 min, the mixture was cooled to 0 °C and H<sub>2</sub>O (80 mL) was cautiously added followed by NaBO<sub>3</sub>•4 H<sub>2</sub>O (4.2 g, 0.027 mol). The mixture was stirred vigorously at rt for 2.3 h, then extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (50% EtOAc-hexanes) to provide alcohol **303** (1.37 g, 87%).

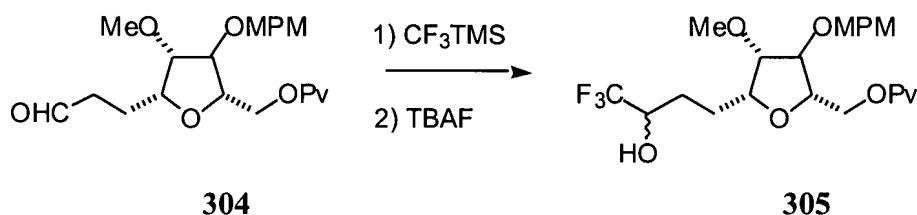


**303**

**304**

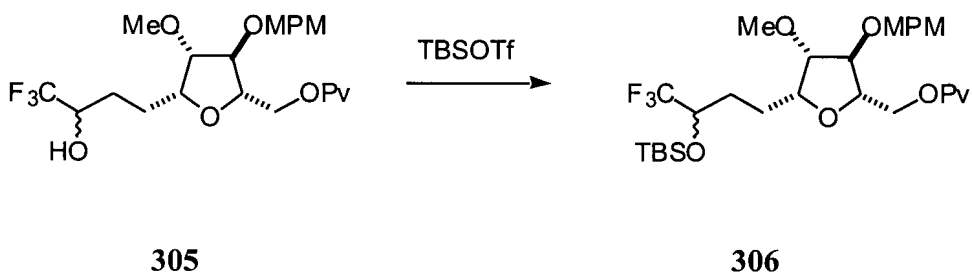
**Aldehyde 304** Oxalyl chloride (88  $\mu$ L, 1.00 mmol) was added dropwise to a solution of DMSO (142  $\mu$ L, 2.00 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL) at  $-78^\circ\text{C}$ . After 30 min, a solution of alcohol **303** (137 mg, 0.335 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 mL) was added and stirred at  $-78^\circ\text{C}$  for 1 h.  $\text{Et}_3\text{N}$  (420  $\mu$ L, 3.01 mmol) was added and after 10 min the reaction was stirred for 10 min at  $0^\circ\text{C}$  at which point saturated aqueous  $\text{NH}_4\text{Cl}$  was added and the resulting mixture was extracted with  $\text{CH}_2\text{Cl}_2$  (3 $\times$ ). The combined organic extracts were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by flash chromatography (50% EtOAc-hexanes) to provide intermediate aldehyde **304** (0.114 g, 84%) which was immediately used in the next step.

10



**Alcohol 305** TBAF (1 M in THF, 5  $\mu$ L, 0.005 mmol) was added to a solution of aldehyde **304** (0.114 g, 0.27 mmol) in  $\text{CF}_3\text{TMS}$  (0.5 M in THF, 1.1 mL, 0.54 mmol) at  $0^\circ\text{C}$ . After 20 min, a second portion of TBAF (1 M in THF, 100  $\mu$ L, 0.1 mmol) was added and the mixture was stirred for 10 min at which point excess TBAF (1 M in THF, 270  $\mu$ L, 0.27 mmol) was added dropwise to cleave the intermediate silyl ether. After 30 min, the mixture was diluted with  $\text{H}_2\text{O}$  and extracted with EtOAc (3 $\times$ ). The organic extracts were washed with  $\text{H}_2\text{O}$ , brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by column chromatography (50% EtOAc-hexanes) to provide alcohol **305** (123 mg, 95%) as an inseparable 1:1 mixture of isomers.

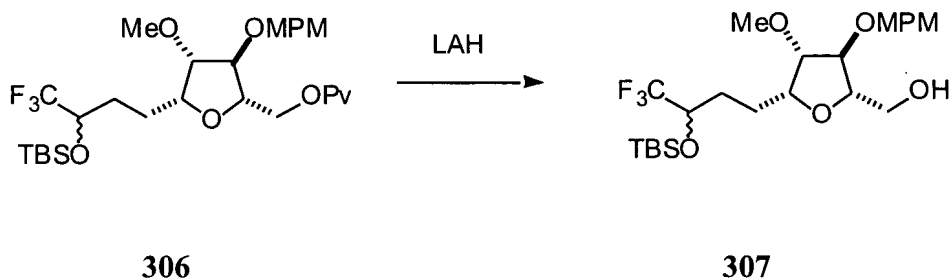
T1030



5

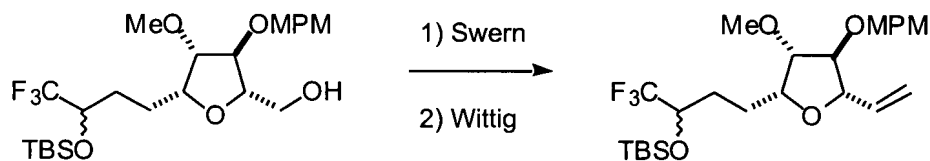
**Silyl ether 306** TBSOTf (265  $\mu\text{L}$ , 1.16 mmol) was added to a solution of alcohol **305** (123 mg, 0.257 mmol) and  $\text{Et}_3\text{N}$  (430  $\mu\text{L}$ , 3.08 mmol) in  $\text{CH}_2\text{Cl}_2$  (8 mL) at 0  $^\circ\text{C}$ . After stirring at rt for 20 h, saturated aqueous  $\text{NaHCO}_3$  was added, and the mixture was extracted with  $\text{CH}_2\text{Cl}_2$  (3 $\times$ ). The combined organic extracts were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide silyl ether **306** (148 mg, 97%).

T1031



15

**Alcohol 307** LAH (1 M in THF, 220  $\mu\text{L}$ , 0.22 mmol) was added dropwise to a solution of silyl ether **306** (131 mg, 0.22 mmol) in  $\text{Et}_2\text{O}$  (5 mL) at 0  $^\circ\text{C}$ . After 20 min,  $\text{H}_2\text{O}$  and 1 M  $\text{NaOH}$  were cautiously added. The mixture was stirred at rt 30 min, filtered through glass wool, concentrated and purified by column chromatography (50% EtOAc-hexanes) to provide alcohol **307** (112 mg, quant.).



11040

**307**

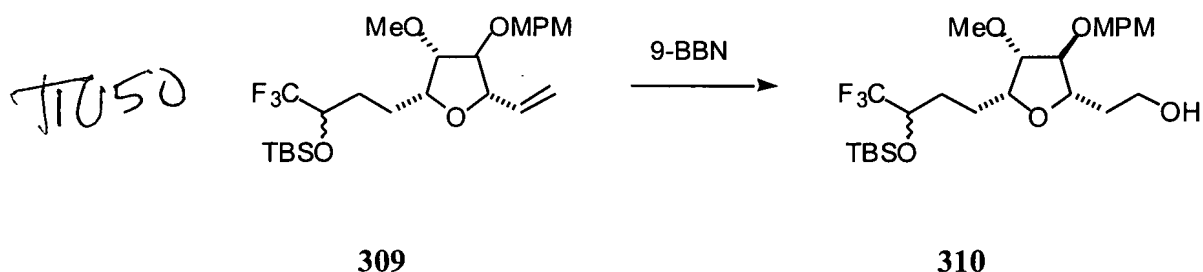
**309**

5        **Alkene 309** Oxalyl chloride (58  $\mu$ L, 0.66 mmol) was added dropwise to a solution of DMSO (94  $\mu$ L, 1.3 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) at  $-78^\circ\text{C}$ . After 30 min, a solution of alcohol **307** (112 mg, 0.22 mmol) in  $\text{CH}_2\text{Cl}_2$  (3 mL) was added. After 1 h,  $\text{Et}_3\text{N}$  (276  $\mu$ L, 1.98 mmol) was added, and after 10 min at  $-78^\circ\text{C}$  the reaction was stirred at  $0^\circ\text{C}$  for 10 min. Saturated aqueous  $\text{NH}_4\text{Cl}$  was added and the mixture was extracted with  $\text{CH}_2\text{Cl}_2$  (3 $\times$ ). The combined organic  
10 extracts were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by flash chromatography (50% EtOAc-hexanes) to provide aldehyde **308** (101 mg, 91%) which was immediately used in the next step.

$n\text{BuLi}$  (1.63 M in THF, 200  $\mu$ L, 0.33 mmol) was added dropwise to a solution of  $\text{CH}_3\text{PPh}_3\text{Br}$  (118 mg, 0.33 mmol) in THF (3 mL) and DMSO (1.2 mL) at  $0^\circ\text{C}$ . After 70 min, a  
15 solution of aldehyde **308** (101 mg, 0.20 mmol) in THF (3 mL) was added and after 10 min at  $0^\circ\text{C}$ , the reaction was stirred at rt for 1 h. Saturated aqueous  $\text{NH}_4\text{Cl}$  was added and the mixture was extracted with EtOAc (3 $\times$ ). The combined organic extracts were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide alkene **309** (90.9 mg, 90%).

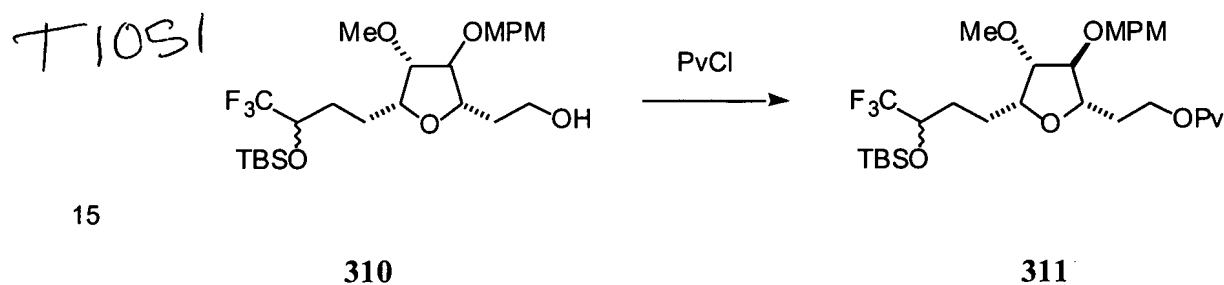
20

104



5

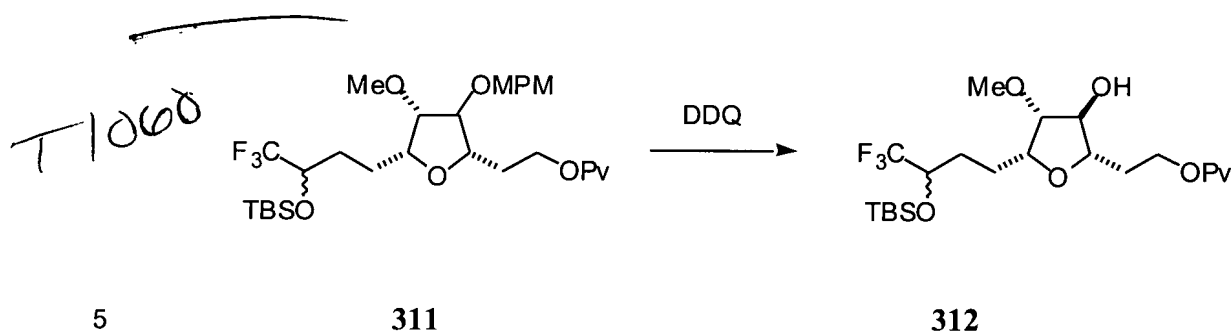
**Alcohol 310** 9-BBN (0.5 M in THF, 17 mL, 8.45 mmol) was added dropwise to a solution of alkene **309** (1.06 g, 2.11 mmol) in THF (30 mL) at 0 °C. After stirring for 2.5 h at rt, the reaction was cooled to 0 °C and H<sub>2</sub>O (60 mL) followed by NaBO<sub>3</sub>•4 H<sub>2</sub>O (3.25 g, 21.1 mmol) were cautiously added. The mixture was stirred vigorously at rt for 2 h, then diluted with H<sub>2</sub>O and extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (20% to 30% EtOAc-hexanes) to provide alcohol **310** (0.920 g, 84%).



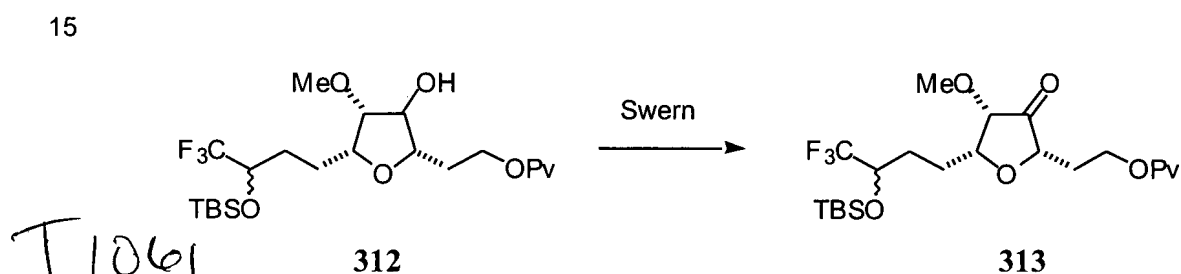
15

**Pivaloate 311** A mixture of alcohol **310** (65.8 mg, 0.0126 mmol), pyridine (61 μL, 0.76 mmol) and PivCl (23 μL, 0.189 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) was stirred at rt for 5 h. A second reaction utilizing alcohol **310** (0.92 g, 1.76 mmol) was run under similar conditions and both reactions were combined during the work-up: saturated aqueous NH<sub>4</sub>Cl was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×). The combined organic extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide pivaloate **311** (1.08 g, quant).



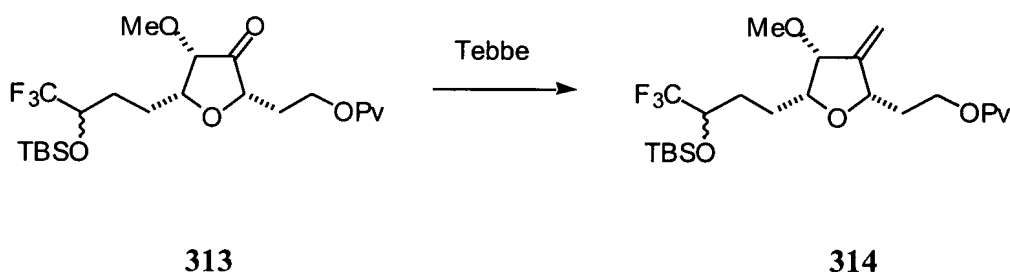


**Alcohol 312** A mixture of ether **311** (0.811 g, 1.33 mmol), DDQ (6.1 g, 27 mmol) and 10:1 *t*BuOH: pH 7 phosphate buffer (42 mL) in CH<sub>2</sub>Cl<sub>2</sub> (84 mL) was stirred vigorously in the dark at rt for 1.5 h, at which point additional DDQ (1.0 g, 4.4 mmol) was added. After 1 h, saturated aqueous NaHCO<sub>3</sub> was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (4×). The combined organic extracts were washed successively with saturated aqueous NaHCO<sub>3</sub> and brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide alcohol **312** (0.56 g, 87%) as well as recovered starting material **311** (97 mg, 12%).

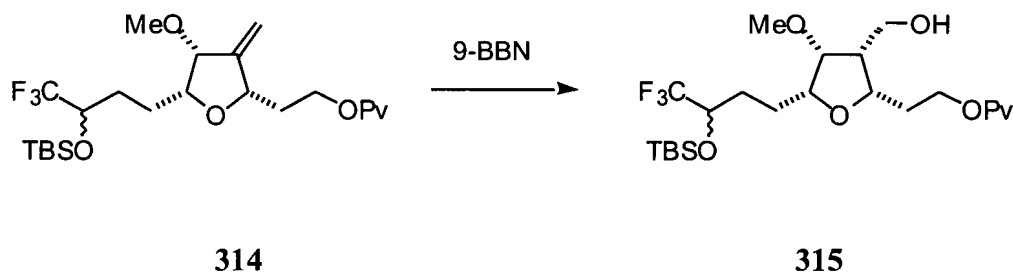


**Ketone 313** Oxalyl chloride (21  $\mu$ L, 0.12 mmol) was added dropwise to a solution of DMSO (34  $\mu$ L, 0.48 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) at -78 °C. After 1 h, a solution of alcohol **312** (39.4 mg, 0.081 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1.5 mL) was added and the mixture was stirred for 1.5 h. Et<sub>3</sub>N (100  $\mu$ L, 0.73 mmol) was added, and after 10 min the mixture was warmed to 0 °C. Saturated aqueous NH<sub>4</sub>Cl was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×). The combined organic extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified

by flash chromatography (30% EtOAc-hexanes) to provide ketone **313** (36.6 mg, 93%) which was used immediately in the next step.



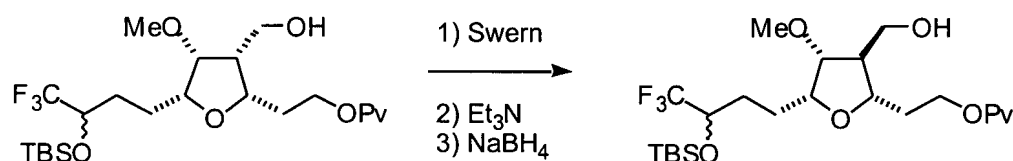
**Alkene 314** Tebbe reagent (~0.65 M in toluene, 720  $\mu$ L, 0.47 mmol) was added dropwise to a solution of ketone **313** (151 mg, 0.31 mmol) in THF (5 mL) at 0  $^{\circ}$ C. After 15 min, H<sub>2</sub>O was cautiously added and the mixture was extracted with EtOAc (3 $\times$ ). The combined organic extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (10% EtOAc-hexanes) to provide alkene **314** (139 mg, 93%).



**Alcohol 315** 9-BBN (0.5 M in THF, 6.0 mL, 2.9 mmol) was added dropwise to a solution of alkene **314** (468 mg, 0.97 mmol) in THF (10 mL) at 0  $^{\circ}$ C. The mixture was stirred at rt for 2 h at which point additional 9-BBN (0.5 M in THF, 500  $\mu$ L, 0.25 mmol) was added. After 2.5 h, the mixture was cooled to 0  $^{\circ}$ C and H<sub>2</sub>O (10 mL) followed by NaBO<sub>3</sub>•4 H<sub>2</sub>O (1.5 g, 9.7 mmol) were cautiously added. The mixture was stirred vigorously at rt for 5 h, diluted with H<sub>2</sub>O

and extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (gradient 20% to 30% EtOAc-hexanes) to provide alcohol **315** (0.47 g, 97%).

5



**315**

**316**

**Alcohol 316** Oxalyl chloride (246 μL, 2.82 mmol) was added dropwise to a solution of DMSO (400 μL, 5.64 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (40 mL) at -78 °C. After 1 h, a solution of alcohol **315** (0.47 g, 0.94 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added and the mixture was stirred for 1 h. Et<sub>3</sub>N (1.2 mL, 8.5 mmol) was added, and after 10 min the mixture was warmed to 0 °C and stirred for 10 min. Saturated aqueous NH<sub>4</sub>Cl was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×). The combined organic extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The crude aldehyde was stirred in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and Et<sub>3</sub>N (2 mL) at rt overnight. Saturated aqueous NH<sub>4</sub>Cl was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×). The combined organic extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by flash chromatography (30% EtOAc-hexanes) provided the epimerized aldehyde which was immediately dissolved in 1:1 Et<sub>2</sub>O:EtOH (10 mL) and cooled to 0 °C. NaBH<sub>4</sub> (35 mg, 0.94 mmol) was added and after 10 min the reaction was quenched with saturated aqueous NH<sub>4</sub>Cl. The mixture was extracted with EtOAc (3×) and the combined organic extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (30% EtOAc-hexanes) to provide alcohol **316** (0.410 g, 87% yield for 3 steps).

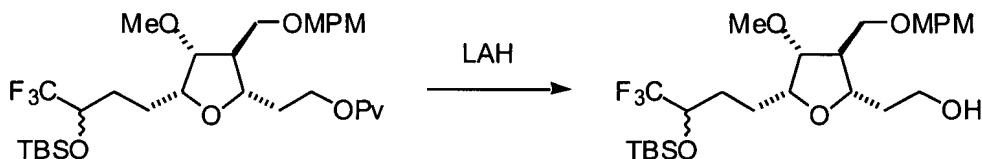
25



316

317

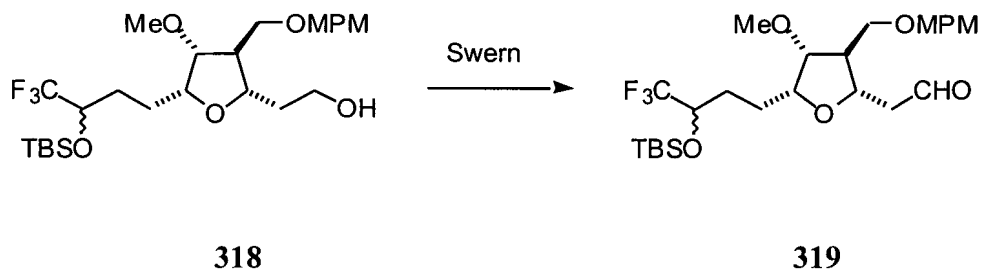
5 **Ether 317** Alcohol **316** (60.7 mg, 0.12 mmol) and MPMOTCI (0.10 g, 0.36 mmol) were combined, azeotroped from toluene (3×) and dried under high vacuum overnight.  $\text{CH}_2\text{Cl}_2$  (3 mL) was added and the mixture was cooled to 0 °C.  $\text{BF}_3 \cdot \text{OEt}_2$  (approx. 1  $\mu\text{L}$ , 0.01 mmol) was added and after stirring for 10 min the reaction was quenched with saturated aqueous  $\text{NH}_4\text{Cl}$ . The mixture was extracted with  $\text{CH}_2\text{Cl}_2$  (3×) and the combined extracts were washed with brine, dried  
10 over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by preparative TLC (30% EtOAc-hexanes) to provide ether **317** (55.4 mg, 74%).



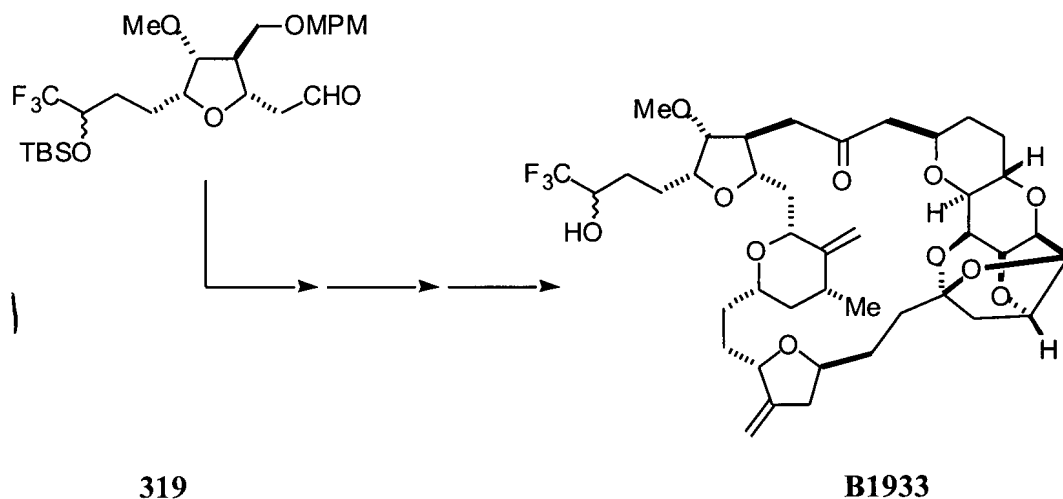
317

318

15 **Alcohol 318** LAH (1 M in THF, 104  $\mu\text{L}$ , 0.104 mmol) was added dropwise to a solution of ether **317** (54 mg, 0.087 mmol) in  $\text{Et}_2\text{O}$  (5 mL) at 0 °C. After 30 min,  $\text{H}_2\text{O}$  and 1 M NaOH were cautiously added. The mixture was stirred at rt for 10 min, filtered through glass wool,  
20 concentrated and purified by column chromatography (30%-50% EtOAc-hexanes) to provide alcohol **318** (45.5 mg, 98%).



- 5 **Aldehyde 319** Oxalyl chloride (11  $\mu$ L, 0.13 mmol) was added dropwise to a solution of DMSO (18  $\mu$ L, 0.25 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL) at  $-78^\circ\text{C}$ . After 1.8 h, a solution of alcohol **318** (22.6 mg, 0.042 mmol) in  $\text{CH}_2\text{Cl}_2$  (1 mL) was added and the mixture was stirred for 1 h.  $\text{Et}_3\text{N}$  (53  $\mu$ L, 0.38 mmol) was added and after 10 min, the reaction was warmed to  $0^\circ\text{C}$  and stirred 10 min. Saturated aqueous  $\text{NH}_4\text{Cl}$  was added and the mixture was extracted with  $\text{CH}_2\text{Cl}_2$  (3 $\times$ ). The
- 10 combined organic extracts were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by flash chromatography (20% EtOAc-hexanes) to provide aldehyde **319** (21.7 mg, 97%).

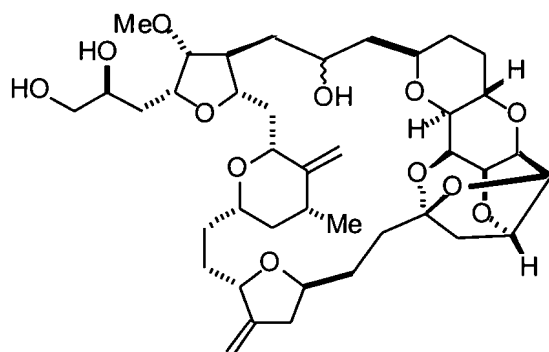


15

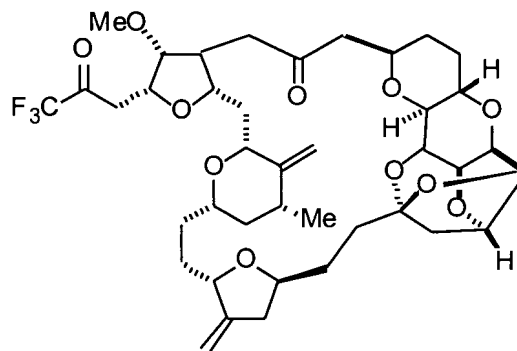
319

B1933

**B1933** In a manner similar to that described in Scheme 6 for the synthesis of **B1794**, intermediate **319** was converted to **B1933**. HRMS (FAB): calcd for  $C_{41}H_{57}F_3O_{11} + H$  783.3931. Found: 783.3940.



1)  $NaIO_4$   
2)  $CF_3TMS$   
3) TBAF  
4) Dess-Martin



**B1897**

**B1942**

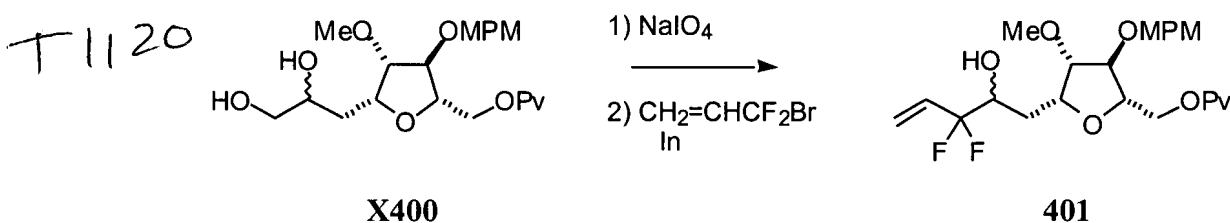
**B1942** A mixture of **B1897** (2 mg, 2.73  $\mu$ mol),  $NaIO_4$  (35 mg, 0.16 mmol), MeOH (0.8 mL) and  $H_2O$  (0.2 mL) was stirred at rt for 30 min. The reaction mixture was then diluted with  $H_2O$  (3 mL) and extracted with  $CH_2Cl_2$  (6 $\times$ ) and EtOAc (2 $\times$ ). The combined organic phases were dried over  $Na_2SO_4$  and purified by column chromatography (5% MeOH-  $CH_2Cl_2$ ) to give the desired aldehyde.

This material was dissolved in THF (0.1 mL), cooled to at 0  $^{\circ}C$  and treated with 0.5 M  $CF_3TMS$  in THF (30  $\mu$ L, 15 mmol) followed by 0.05 M TBAF in THF (5 mL, 0.025 mmol). After stirring for 30 min, the reaction mixture was diluted with saturated aqueous  $NaHCO_3$  (2 mL) and  $H_2O$  (1 mL), extracted with EtOAc (6 $\times$ ), dried over  $Na_2SO_4$ , filtered and concentrated to give the crude bis-TMS ether.

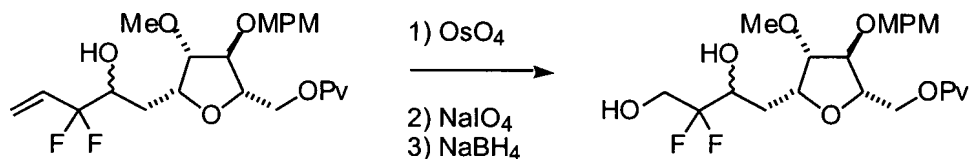
This material was dissolved in THF (0.5 mL) and treated with 1 M TBAF in THF containing 0.5 M imidazole hydrochloride (8  $\mu$ L, 8  $\mu$ mol) at rt for 30 min. The reaction mixture was eluted through a SiO<sub>2</sub> column (50% EtOAc-hexanes to EtOAc) to afford the diol intermediate.

- 5 A mixture of this product and Dess-Martin periodinane (10 mg, 24 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.5 mL) was stirred at rt for 1 h, diluted with Et<sub>2</sub>O (5 mL) and filtered through Celite. The filtrate was concentrated and purified by preparative TLC (50% EtOAc-hexanes) to furnish **B1942** (1.5 mg, 72% for 5 steps). HRMS (FAB): calcd for C<sub>40</sub>H<sub>55</sub>F<sub>3</sub>O<sub>11</sub> + H 767.3516. Found: 767.3542

#### 10 Synthesis of B2070/B2073:



- 15 **Alcohol 401** A mixture of NaIO<sub>4</sub> (375 mg, 1.74 mmol), **X400** (674 mg, 1.58 mmol), MeOH (16 mL) and H<sub>2</sub>O (4 mL) was stirred at rt for 1 h. After dilution with H<sub>2</sub>O, the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (4 $\times$ ) and the combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by flash chromatography (30% EtOAc-hexanes) to provide the intermediate aldehyde (570 mg), which was immediately dissolved in DMF (15 mL). Indium
- 20 (275 mg, 2.4 mmol) and 3-bromo-3,3-difluoropropene (240  $\mu$ L, 2.4 mmol) were added and after stirring at rt for 17 h, H<sub>2</sub>O and 0.1 M HCl were added. The mixture was extracted with EtOAc (3 $\times$ ) and the combined organic extracts were washed successively with H<sub>2</sub>O and brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (20% to 30% EtOAc-hexanes) to provide alcohol **401** as a 1:1 mixture of C34 isomers (605 mg, 81% for 2 steps).



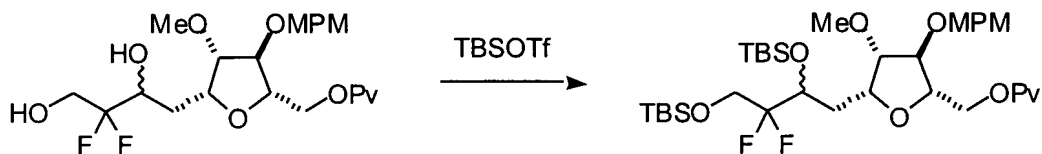
401

402

5 **Diol 402** A mixture of OsO<sub>4</sub> (1 xstal), alcohol **401** (605 mg, 1.28 mmol), 4-methylmorpholine *N*-oxide (0.45 g, 3.84 mmol), acetone (30 mL) and H<sub>2</sub>O (6 mL) was stirred at rt for 29 h. Additional OsO<sub>4</sub> (3 xtals) and 4-methylmorpholine *N*-oxide (0.1 g, 0.8 mmol) were added and after 2 days saturated aqueous Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> was added. The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (6×) and the combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The crude

10 intermediate triol was immediately dissolved in 4:1::MeOH:H<sub>2</sub>O (25 mL) and NaIO<sub>4</sub> (0.41 g, 1.9 mmol) was added. After stirring vigorously at rt for 2 h, the mixture was diluted with H<sub>2</sub>O, extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×) and the combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to provide the intermediate aldehyde which was immediately dissolved in 1:1 EtOH-Et<sub>2</sub>O (30 mL) and cooled to 0 °C. NaBH<sub>4</sub> (48 mg, 1.3 mmol) was added and after 20 min

15 the reaction was quenched with H<sub>2</sub>O and extracted with CH<sub>2</sub>Cl<sub>2</sub> (4×). The combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (50% EtOAc-hexanes) to provide diol **402** (485 mg, 80% for 3 steps).

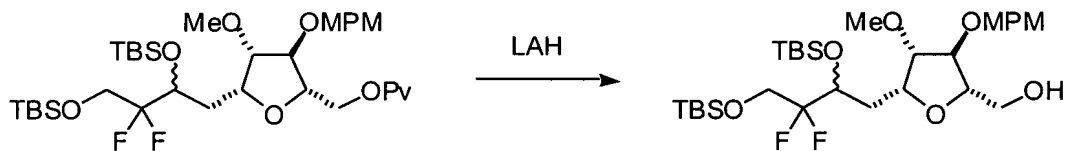


402

403



**Silyl ether 403** TBSOTf (2.3 mL, 10 mmol) was added dropwise to a mixture of diol **402** (485 mg, 1.0 mmol), Et<sub>3</sub>N (2.8 mL, 20 mmol) and CH<sub>2</sub>Cl<sub>2</sub> (30 mL) at 0 °C. After stirring for 1 h at rt, saturated aqueous NH<sub>4</sub>Cl was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×). The combined organic extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide silyl ether **403** (668 mg, 95%).



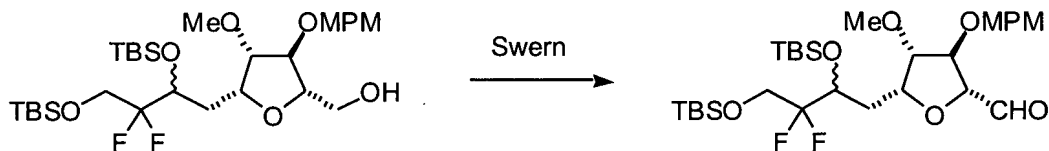
10

**403**

**4**

**04**

**Alcohol 404** LAH (1 M in THF, 2.8 mL, 2.8 mmol) was added dropwise to a solution of silyl ether **403** (668 mg, 0.948 mmol) in Et<sub>2</sub>O (60 mL) at 0 °C. After 15 min, H<sub>2</sub>O and 1 M NaOH were cautiously added. The mixture was stirred at rt for 20 min, filtered through glass wool, concentrated and purified by column chromatography (30% EtOAc-hexanes) to provide alcohol **404** (500 mg, 85%).



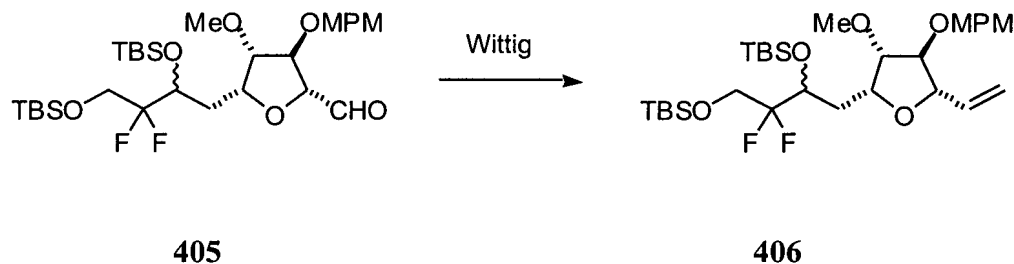
20

**404**

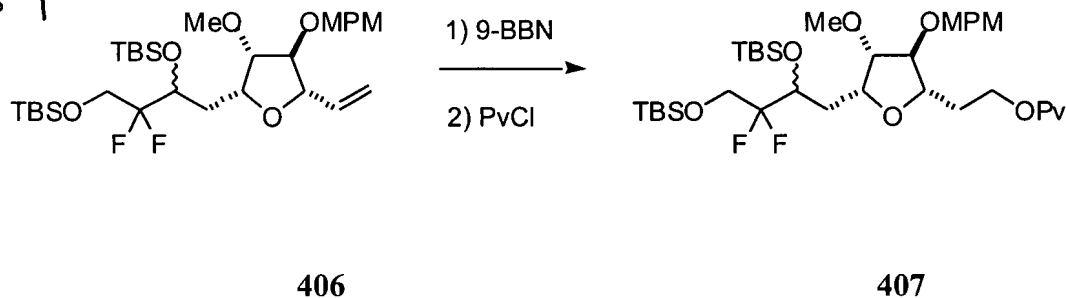
**405**

**Aldehyde 405** Oxalyl chloride (210 µL, 2.42 mmol) was added dropwise to a solution of DMSO (345 µL, 4.84 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) at -78 °C. After 1 h, a solution of alcohol **404**

(500 mg, 0.806 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) was added. After 40 min,  $\text{Et}_3\text{N}$  (1.0 mL, 7.2 mmol) was added. After stirring at  $-78^\circ\text{C}$  for 10 min, the reaction mixture was warmed to  $0^\circ\text{C}$  and stirred for an additional 10 min. Saturated aqueous  $\text{NH}_4\text{Cl}$  was added and the mixture was extracted with  $\text{CH}_2\text{Cl}_2$  (3 $\times$ ). The combined organic extracts were washed successively with  $\text{H}_2\text{O}$ ,  
 5 brine, dried over  $\text{Na}_2\text{SO}_4$  and concentrated. Purification by flash chromatography (30% EtOAc-hexanes) provided aldehyde **405** (486 mg, 98%) which was immediately used in the next step.



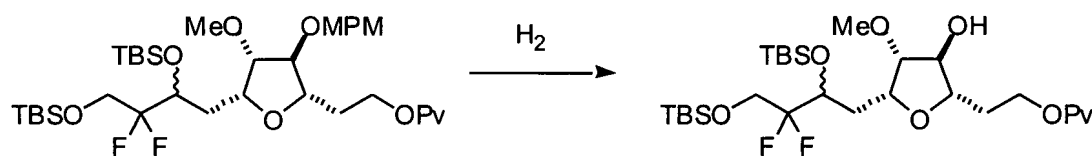
**Alkene 406**  $n\text{BuLi}$  (1.63 M, 860  $\mu\text{L}$ , 1.4 mmol) was added dropwise to a solution of  $\text{CH}_3\text{PPh}_3\text{Br}$  (500 mg, 1.4 mmol) in THF (15 mL) and DMSO (6 mL) at  $0^\circ\text{C}$ . After 1 h, a solution of aldehyde **405** (486 mg) in THF (15 mL) was added. The reaction mixture was  
 15 warmed to rt and stirred for 30 min. Saturated aqueous  $\text{NH}_4\text{Cl}$  was added, the mixture was extracted with EtOAc (3 $\times$ ) and the combined extracts were washed successively with  $\text{H}_2\text{O}$  and brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide alkene **406** (450 mg, 93%).



**Ester 407** 9-BBN (0.5 M in THF, 9.0 mL, 4.5 mmol) was added dropwise to a solution of alkene **406** (0.460 g, 0.746 mmol) in THF (10 mL) at 0 °C. After warming to rt, the mixture was stirred for 3 h and two additional portions of 9-BBN (0.5 M in THF, 3.0 mL, 1.5 mmol) were added at 30 min intervals. The reaction mixture was recooled to 0 °C, whereupon THF

5 (10 mL), H<sub>2</sub>O (10 mL) and NaBO<sub>3</sub>•4 H<sub>2</sub>O (1.72 g, 11.2 mmol) were cautiously added. The mixture was stirred vigorously at rt for 1.5 h, and additional NaBO<sub>3</sub>•4 H<sub>2</sub>O (1.0 g, 6.5 mmol) was added. After 2 h the mixture was diluted with H<sub>2</sub>O and extracted with EtOAc (3×). The combined extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (20% to 30% EtOAc-hexanes) to provide the intermediate alcohol (509  
10 mg) which was immediately dissolved in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and treated with pyridine (600 μL, 7.5 mmol) and PvCl (275 μL, 2.2 mmol). After 6 h, saturated aqueous NH<sub>4</sub>Cl was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×). The combined organic extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (20% to 30% EtOAc-hexanes) to provide ester **407** (423 mg, 79% for 2 steps).

15

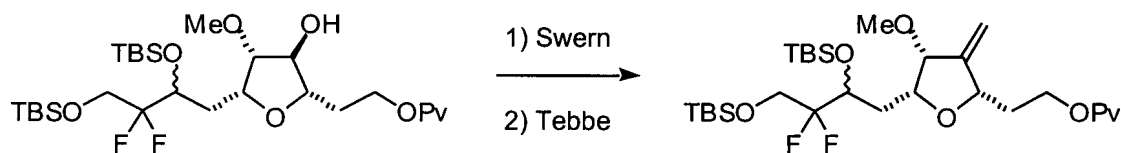


**407**

**408**

**Alcohol 408** A mixture of ester **407** (11 mg, 0.015 mmol) and Pd(OH)<sub>2</sub>/C (10 mg) in EtOAc (500 μL) was stirred vigorously under a H<sub>2</sub> atmosphere at rt for 6 h. The mixture was filtered through Celite, concentrated and purified by column chromatography (30% EtOAc-hexanes) to provide alcohol **408** (9.4 mg, quant).

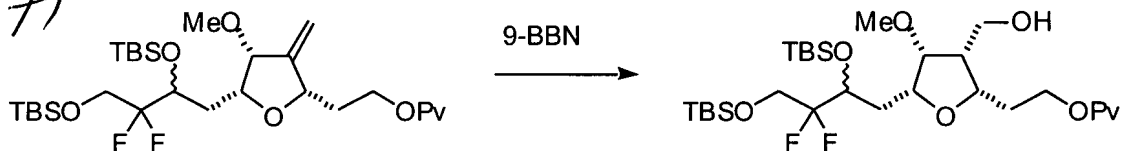
20



408

409

**Alkene 409** Oxalyl chloride (7  $\mu$ L, 0.075 mmol) was added dropwise to a solution of  
 5 DMSO (11  $\mu$ L, 0.15 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL) at  $-78^\circ\text{C}$  under  $\text{N}_2$ . After 40 min, a solution of  
 alcohol **408** (15.2 mg, 0.025 mmol) in  $\text{CH}_2\text{Cl}_2$  (1 mL) was added and the reaction was stirred at  $-$   
 $78^\circ\text{C}$  for 1 h.  $\text{Et}_3\text{N}$  (31  $\mu$ L, 0.22 mmol) was added, and after stirring for 10 min the mixture was  
 warmed to  $0^\circ\text{C}$ . After 10 min, the reaction mixture was quenched with saturated aqueous  $\text{NH}_4\text{Cl}$   
 and extracted with  $\text{CH}_2\text{Cl}_2$  (3 $\times$ ). The combined extracts were washed successively with  $\text{H}_2\text{O}$  and  
 10 brine, dried over  $\text{Na}_2\text{SO}_4$  and concentrated. After flash chromatography (30% EtOAc-hexanes),  
 the intermediate ketone (13 mg) was immediately dissolved in THF (500  $\mu$ L) and treated with  
 Tebbe reagent ( $\sim 0.65$  M in toluene, 62  $\mu$ L, 0.040 mmol) at  $0^\circ\text{C}$ . After 1.5 h additional Tebbe  
 reagent ( $\sim 0.65$  M in toluene, 62  $\mu$ L, 0.040 mmol) was added and after 10 min  $\text{H}_2\text{O}$  and then  
 brine were cautiously added. The mixture was extracted with EtOAc (3 $\times$ ) and the combined  
 15 organic extracts were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , concentrated and purified by  
 column chromatography (10% EtOAc-hexanes) to provide alkene **409** (11.9 mg, 80% for 2  
 steps).

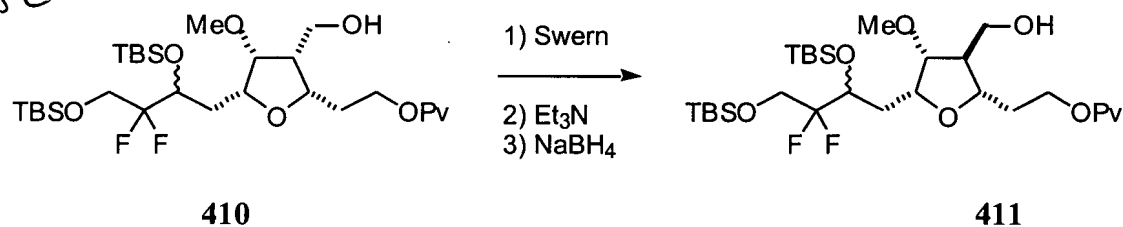


409

410

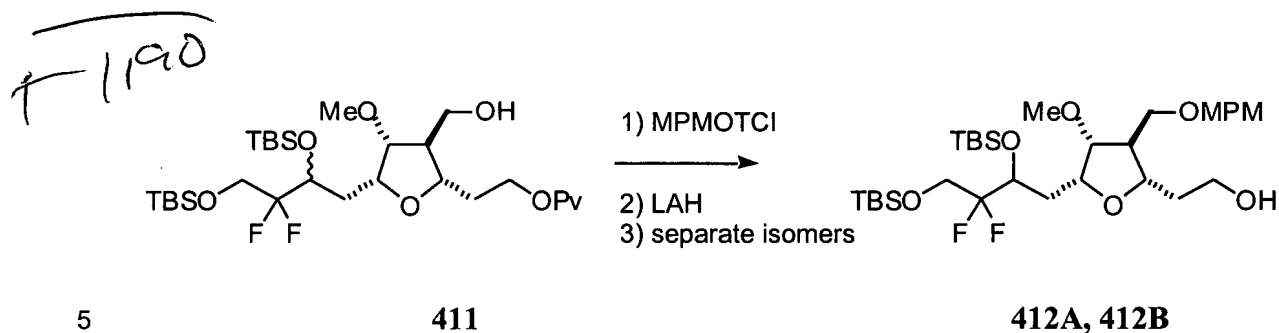
20

**Alcohol 410** 9-BBN (0.5 M in THF, 1.5 mL, 0.72 mmol) was added dropwise to a solution of alkene **409** (0.144 g, 0.242 mmol) in THF (2 mL) at 0 °C. After warming to rt, the mixture was stirred for 3 h. The reaction mixture was recooled to 0 °C, whereupon THF (2 mL), H<sub>2</sub>O (2 mL) and NaBO<sub>3</sub>•4 H<sub>2</sub>O (0.38 g, 2.4 mmol) were cautiously added. The mixture was stirred vigorously at rt for 4 h, diluted with H<sub>2</sub>O and extracted with EtOAc (3×). The combined extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide alcohol **410** (0.140 g, 94%).



**Alcohol 411** Oxalyl chloride (26 µL, 0.30 mL) was added dropwise to a solution of DMSO (43 µL, 0.60 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (4 mL) at -78 °C. After 1 h, a solution of alcohol **410** (57 mg, 0.093 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) was added. After 45 min, Et<sub>3</sub>N (125 µL, 0.90 mmol) was added. After stirring at -78 °C for 10 min, the reaction mixture was warmed to 0 °C and stirred for an additional 10 min. Saturated aqueous NH<sub>4</sub>Cl was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×). The combined organic extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The crude product was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (4 mL), treated with Et<sub>3</sub>N (400 µL) and stirred at rt for 15 h. Saturated aqueous NH<sub>4</sub>Cl was added and the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×). The combined organic extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by flash chromatography (30% EtOAc-hexanes) to provide the intermediate aldehyde (48 mg), which was immediately dissolved in 1:1 Et<sub>2</sub>O-EtOH (4 mL), cooled to 0 °C and treated with solid NaBH<sub>4</sub> (~4 mg, 0.09 mmol). After stirring for 15 min, saturated aqueous NH<sub>4</sub>Cl was cautiously added and the mixture was extracted with EtOAc (3×). The combined extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by

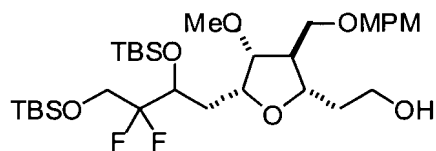
column chromatography (20% to 30% EtOAc-hexanes) to provide alcohol **411** (45.6 mg, 80% for 3 steps).



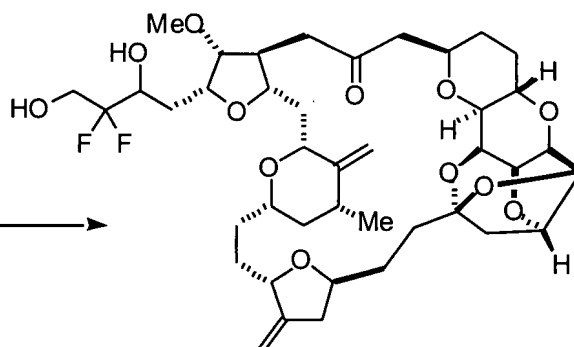
**412A and 412B** Alcohol **411** (120 mg, 0.196 mmol) and MPMOTCl (0.17 g, 0.59 mmol) were combined, azeotroped from toluene (3×) and dried under high vacuum for 1 h. CH<sub>2</sub>Cl<sub>2</sub> (9 mL) was added and the mixture was cooled to 0 °C. BF<sub>3</sub>•OEt<sub>2</sub> (0.016 M in CH<sub>2</sub>Cl<sub>2</sub>, 125 µL, 0.002 mmol) was added dropwise and after stirring for 20 min, the reaction was

10 quenched with saturated aqueous NH<sub>4</sub>Cl. The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×) and the combined extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by preparative TLC (20% EtOAc-hexanes) to provide the intermediate MPM ether which contained some close-running impurities. This material was immediately dissolved in Et<sub>2</sub>O (10 mL) and treated with LAH (1M in THF, 300 µL, 0.300 mmol) at 0 °C. After 10 min, H<sub>2</sub>O and 1 M NaOH

15 were added, and after stirring for 10 min at rt, the mixture was filtered through Celite, concentrated and purified by preparative TLC (35% EtOAc-hexanes) to provide **412A** (49 mg, 39% for 2 steps) as a single C34 isomer and **412B** (46 mg, 36% for 2 steps) as a ~9:1 mixture of C34 isomers.



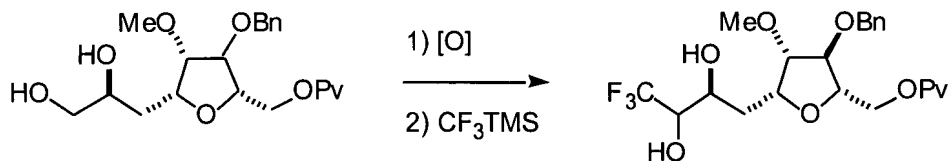
412A, 412B



B2070, B2073

**B2070 and B2073** In a manner similar to that described in Schemes 4 and 6 for the synthesis of **B1794**, intermediates **412A** and **412B** were converted to **B2070** and **B2073**, respectively. For **B2070**: HRMS (FAB): calcd for  $C_{41}H_{58}F_2O_{12} + Na$  803.3794. Found: 803.3801. For **B2073**: HRMS (FAB): calcd for  $C_{41}H_{58}F_2O_{12} + Na$  803.3793. Found: 803.3781

#### Synthesis of B1963:



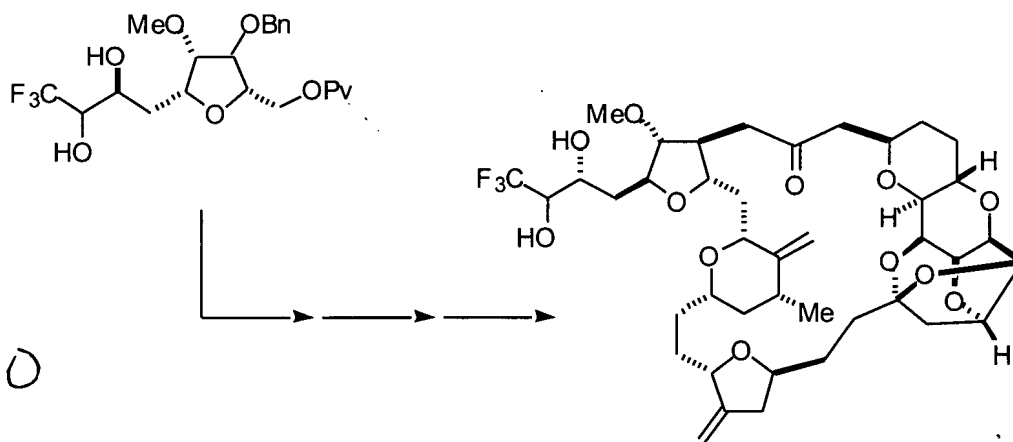
10

501

**Diol 501 (64)** Saturated aqueous  $NaHCO_3$  (21 mL) and KBr (89 mg, 0.75 mmol) were added to a solution of diol **10** (1.35 g, 3.4 mmol) in  $CH_2Cl_2$  (34 mL). The mixture was cooled to 0 °C, and 4-methoxy-2,2,6,6-tetramethyl-1-piperidinyloxy (0.05 M in  $CH_2Cl_2$ , 7.45 mL, 0.37 mmol) and  $NaOCl$  (0.07 M in  $H_2O$ , 5.6 mL, 0.39 mmol) were sequentially added. After 1 h, the reaction mixture was quenched with saturated aqueous  $Na_2S_2O_3$ , diluted with saturated aqueous

NaHCO<sub>3</sub> and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3×). The combined extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and dissolved in THF (21 mL).

After cooling to 0 °C, CF<sub>3</sub>TMS (1.5 g, 10.5 mmol) and TBAF (0.1 M in THF, 680 μL, 0.068 mmol) were sequentially added. After stirring for 40 min, additional TBAF (1 M in THF, 8.3 mL, 8.3 mmol) was added. After 30 min, the reaction was quenched with H<sub>2</sub>O and extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated and purified by flash chromatography (30%, 40%, 50% EtOAc-hexanes followed by EtOAc) to afford a 2:1 mixture of diols (553 mg, 35%). Separation by MPLC (1.5% MeOH-CH<sub>2</sub>Cl<sub>2</sub>) gave the major, more polar isomer **501 (64)** (340 mg, 22%) and the minor, less polar isomer (152 mg, 10%).

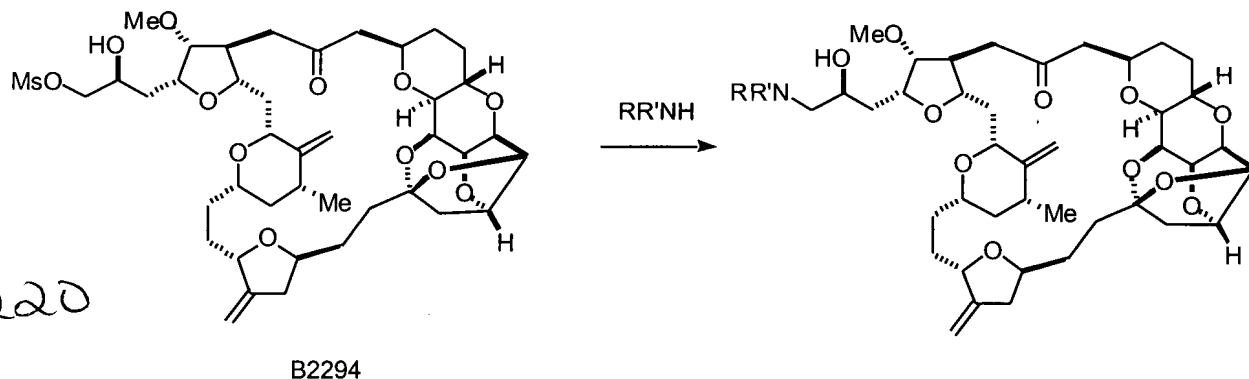


**B1963**

**B1963** In a manner similar to that described in Schemes 4 and 6 for the synthesis of **B1794**, intermediate **501** was converted to **B1963**.



## Synthesis of B2320 and Related Analogs



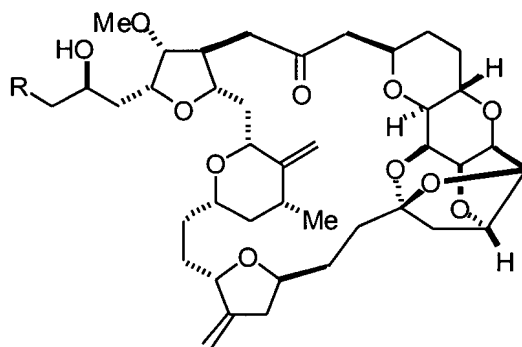
5 These compounds are made by treating B2294 with an appropriate amine in a solvent such as methanol for a period of a few hours to several days. Progress of the reaction may be monitored by thin layer chromatography. A standard work-up procedure, well known to those of skill in the art, provides the desired compounds. The procedure below is to prepare ER803868; however this procedure is general and can be used to prepare any desired analog.

10

### Synthesis of ER803868

To a solution of B2294, 1.2 mg, in methanol, 0.5 mL, was added morpholine, 0.012 mL. The mixture was stirred for 10 days with additional morpholine, 0.012 mL, being added on days 1,2,3, 4 and 8. The mixture was then chromatographed to give 1.4 mg of the desired compound.

15



B2320	R = N,N-dimethylamino
B2330	R = N-isopropylamino
B2336	R = N-methylamino
B2339	R = N-t-butylamino
B2417	R = N-2-hydroxyethylamino
B2418	R = N-piperazinyl
B2489	R = N,N-bis-(2-hydroxyethyl)amino
B2490	R = N-1,3-dihydroxy-2-propylamino
B2491	R = N-benzylamino
ER803834	R = N-piperidinyl
ER803835	R = N-pyrrolidinyl
ER803836	R = N-3-(R)-hydroxypyrrolidinyl
ER803843	R = N-homopiperidinyl
ER803845	R = N-para-methoxybenzylamino
ER803846	R = N-phenethylamino
ER803851	R = N-2-(S-hydroxymethyl)pyrrolidinyl
ER803852	R = N-2-(R-hydroxymethyl)pyrrolidinyl
ER803868	R = N-morpholinyl
ER803869	R = N-ethylamino
ER803870	R = N-imidazolyl
ER803883	R = N,N-diethylamino
ER803884	R = N-para-chlorobenzylamino

#### D. Pharmacological Activity

Many of the individually disclosed drugs were tested for *in vitro* and *in vivo* activity (see Table 1, below). Screening methods included a standard *in vitro* cell growth inhibition assay using DLD-1 human colon cancer cells (ATCC accession number CCL 221) in a 96-well

microtiter plate format (Finlay, G.J. et al Analytical Biochemistry 139:272-277, 1984), a U937 (ATCC accession number CRL 1593) mitotic block reversibility assay (described below), and in some cases, a LOX human melanoma tumor xenograft *in vivo* growth inhibition assay (see Table 1). Chemical stability to esterase degradation was also examined.

#### 5 U937 Mitotic Block Reversibility Assay

U937 human histiocytic lymphoma cells were added to 75 cm<sup>2</sup> tissue culture flasks as 2.5 x 10<sup>6</sup> cells in 22.5 mL of RPMI Medium 1640 containing 10% Fetal Bovine Serum. Cells were allowed to adapt to the culture during 36 h of incubation at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>. Each test drug was then added to a flask as 2.5 mL of 10x final  
10 concentration. Final concentrations achieved were 0.1-1000 nM, in half log-increments, for a total of 10 concentration steps including a drug-free control flask which recieved 2.5 mL of media. Cells were incubated with drug for 12 h pretreatment period at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>.

The contents were removed from each flask and centrifuged at 300 x g for 10 min at room  
15 temperature, after which drug-containing media was removed from cell pellet. Cells were resuspended in 25 mL of warm drug-free media and centrifuged at 300 x g for 10 min at room temperature. After removing media from cell pellet, cells were resuspended in 35 mL of warm drug-free media, transferred to fresh flasks, and a 10 mL sample of cells immediately removed from each flask, immediately processed as described below and stored for later cell cycle  
20 analysis (0 hours of drug washout).

Incubation of the remaining 25 mL of cells continued in drug-free media for another 10 h. A 10 mL sample of cells was removed from each flask, immediately processed and stored for later cell cycle analysis (10 hours of drug washout) and 10 mL fresh replacement media was added to each incubation flask. Incubation of cells in drug-free media continued for 5 days. At  
25 day two, 20 mL of media and cells was removed from each flask and replaced with 20 mL fresh

media. Viability of cells was quantified after 5 days by trypan blue exclusion techniques using hemacytometer counting.

Cells were processed for cell cycle analysis using modifications of the method published in Becton Dickinson Immunocytometry Systems source book section 1.11 (Preparation of  
5 Alcohol-Fixed Whole Cells From Suspensions For DNA Analysis). Briefly, each 10 mL sample of cells removed from the flasks at 0 and 10 hours of drug washout was separately centrifuged at 300 x g for 10 min. After removing the media from the cell pellet, cells were resuspended in 3 mL cold saline. Seven milliliters cold 100 % ethanol was slowly added with vigorous vortexing. Ethanol treated cell samples from 0 hour and 10 hour periods of compound washout were stored  
10 overnight at 4 °C. Ethanol treated cells were centrifuged 300 x g for 10 min, ethanol removed and cells then washed in 10 mL Phosphate Buffered Saline (PBS). Cells were resuspended in 0.5 mL of 0.2 mg/mL Ribonuclease A (Sigma No. R-5503) in PBS and incubated in 37 °C water bath for 30 min.

Cells were transferred to appropriate flow cytometry tubes and 0.5 mL of 10 mg/mL  
15 propidium iodide (PI) (Sigma No. P4170) in PBS was added to each tube. Cells were incubated with PI at room temperature in the dark for at least 15 min prior to analysis with a flow cytometer (Becton Dickinson FACScan flow cytometer or equivalent). Cells should be analyzed within an hour and kept in the dark at 4 °C until ready. Cell cycle analysis was performed on 0 hour and 10 hour cells using flow cytometric measurement of the intensity of cellular  
20 fluorescence. The intensity of propidium iodide fluorescence for each cell was measured on a linear amplification scale with doublet events ignored using doublet discrimination. The results obtained from analyzing 15,000 cells were presented as a histogram with increasing fluorescence intensity on the x-axis and the number of cells at a particular intensity level on the y-axis.

The intensity of PI staining is dependent on the amount of DNA in the cell so it is  
25 possible to identify cells in various phases of the cell cycle, such as cells that have not yet synthesized DNA since the last mitosis (G<sub>1</sub> phase), cells that are in intermediate stages of DNA synthesis (S phase), and cells that have doubled their complement of DNA and are ready to

divide ( $G_2$  phase). Cells that are blocked in the mitosis phase of the cell cycle also have double the amount of DNA compared to  $G_1$  phase cells. If all cells are blocked in mitosis there are no  $G_1$  phase cells, but if the block is removed when compound is removed, cells complete mitosis and reappear in the  $G_1$  phase. The number of cells so reappearing in the  $G_1$  or Sphase is thus a measure of the number of cells which have recently completed mitosis. For each sample at 0 and 10 hours after compound removal, the percentage of cells completing mitosis was quantified (as the number of cells reappearing in the  $G_1$  phase) and plotted as a function of the initial concentration of compound used during the 12 hour pretreatment period. The percentage of cells still viable 5 days after drug washout was superimposed on the same graph, ~~see, for example~~ ~~FIG. 1 and FIG. 2.~~ A ratio can be determined between the compound concentration required to completely block all cells in mitosis at 0 hour and the concentration required to maintain the block 10 hours after compound removal. This was taken as a measure of a compound's reversibility, with ratios close to or equal to one indicating likely potent *in vivo* anti-tumor compounds (see Table 1, columns 4-6, and ~~FIGS. 3 and 4~~).

15

Table 1

**In Vitro Inhibition and Reversibility Data**

Compound	DLD-1*		Complete Mitotic Block		Reversibility
	mean IC <sub>50</sub> , nm	SE	0 hour, nM**	10 hour, nM‡	Ratio
B1793	0.93	0.04	3	44	14.7
B1794	12.20	0.72			
B1918	1.27	0.12			
B1920	2.00	0.15			
B1921	24.00	1.15			
B1922	0.53	0.01	3	30	10.0
B1930	0.87	0.03			
B1933	0.79	0.16			
B1934	1.05	0.21	3	30	10.0
B1939	19.34	2.36	12	12	1.0
B1940	5.43	0.62			
B1942	0.60	0.03	3	30	10.0

B1963	0.56	0.04	3	20	6.7
B1973	1.15	0.24			
B1984	1.01	0.15			
B1987	1.82	0.21			
B1988	2.67	1.02			
B1990	1.30	0.06			
B1991	0.69	0.03			
B1992	0.86	0.07			
B1998	1.23	0.13			
B2003	1.21	0.12			
B2004	0.63	0.04			
B2008	2.63	0.63			
B2010	0.71	0.12			
B2011	1.81	0.52			
B2013	0.49	0.07	2	30	15.0
B2014	0.87	0.09			
B2015	2.78	0.23			
B2016	0.66	0.06			
B2019	0.82	0.07			
B2034	0.74	0.03			
B2035	0.76	0.09			
B2037	0.66	0.11			
B2039	0.91	0.08			
B2042	1.93	0.11	3	100	33.3
B2043	1.70	0.06			
B2070	0.64	0.09	3	30	10.0
B2073	0.89	0.15			
B2086	11.17	1.96			
B2088	1.23	0.12			
B2090	0.52	0.04	2	10	5.0
B2091	1.36	0.07	3	30	10.0
B2102	3.47	0.22			
B2136	5.23	1.04	3	3	1.0
B2294	0.80	0.01			
B2320	1.20	0.17	1	10	10.0
B2330	4.40	0.42	7	7	1.0

B2336	3.33	0.09	10	10	1.0
B2339	4.30	0.21	3	3	1.0
B2417	12.67	0.33	10	10	1.0
B2418	3.63	0.17	10	10	1.0
B2489	14.67	2.03	10	10	1.0
B2490	35.67	3.33	100	100	1.0
B2491	0.92	0.14	2	10	6.7
ER803834	0.47	0.08	1	10	10.0
ER803835	15.33	0.33	10	10	1.0
ER803836	1.97	0.12	3	10	3.3
ER803843	0.49	0.05	1	10	10.0
ER803845	1.50	0.20	3	10	3.3
ER803846	1.16	0.10	1	10	10.0
ER803851	3.33	0.26	3	3	1.0
ER803852	3.03	0.50	3	10	3.3
ER803868	0.43	0.03	3	10	3.3
ER803869	4.13	0.64	3	3	1.0
ER803870	1.27	0.12	3	30	10.0
ER803883	1.02	0.04	1	10	10.0
ER803884	0.59	0.03	1	10	10.0

\* = in vitro cell growth inhibition \*\* = before washout ‡ = after washout

The invention also features a method for identifying an agent that induces a sustained mitotic block in a cell after transient exposure of the cell to the agent. The invention features determining the relative reversibility of the test compound by relating the measurement of step (d) and the measurement of step (f), as described below. This determination may be a ratio, or an arithmetic difference, for example. In one aspect, the method includes:

(a) incubating a first cell sample with a predetermined concentration of a test compound for a time interval between that sufficient to empty the  $G_1$  population and that equivalent to one cell cycle (e.g., typically, 8 –16 hours, or about 12 hours);

(b) substantially separating the test compound from said first cell sample (e.g. by washing or changing media);

(c) incubating said first sample in media free of the test compound for a time interval sufficient to allow at least 80% (e.g., 85%, 90%, and preferably 95%, 98%, or 99%) of the cells released from the mitotic block induced by a highly reversible mitotic inhibitor to complete mitosis and return to the G<sub>1</sub> phase (e.g., typically 6-14 hours, or about 10 hours after separation step (b)); and

5

(d) measuring the percentage of transiently-exposed cells from step (c) that have completed mitosis and returned to the G<sub>1</sub> phase (e.g., measuring a cell cycle marker, such as DNA-dependent PI fluorescence).

10           One aspect of this screening method include the further steps of :

(e) incubating a second sample of cells with a concentration of the test compound less than or equal to that used in step (a) for a time interval between that sufficient to empty the G<sub>1</sub> population and that equivalent to one cell cycle;

15

(f) measuring the percentage of cells from step (e) that have completed mitosis and have returned to the G<sub>1</sub> phase; and

(g) determining a reversibility ratio of the test compound.

20

In one embodiment of the method, the first and second cell samples are suspension culture cells selected from, for example, human leukemia, human lymphoma, murine leukemia, and murine lymphoma cells. The first and second cell samples may be incubated simultaneously (steps (a) and (e)) or in separate portions. Other embodiments further include before step (a), the step (i) of estimating a desirable time interval for incubating said first cell sample with a reversible mititotic blocking agent (or, alternatively, said test compound) to provide a satisfactory majority of cells collected at mitotic block; and wherein the incubation of step (a) is for the time interval estimated in step (i). Another embodiment of the method further includes before step (c), the step (ii) of estimating a desirable time interval for the test compound-free incubation of step (c), said step (ii) comprising determining the time interval after which at least 80 % of the cells pretreated with a highly reversible antimitotic agent complete mitosis and reenter G<sub>1</sub> phase; and wherein the incubation of step (c) is for the time interval determined in step

25

30



(ii). Another embodiment of the method utilizes non-suspension culture cells from, for example, adherent human or murine cancer cells, harvested by any suitable means for detaching them from tissue culture flasks.

One aspect of the method further includes repeating steps (a) – (f) using a range of  
5 relative concentrations of test compound to determine what two substantially minimum  
concentrations of the test compound provide substantially complete mitotic block in step (d) and  
in step (f), respectively. The ratio of these minimum sufficient concentrations is an index of  
reversibility (see detailed U937 protocol for preparation of exemplary dose-response curves).  
These concentrations may be determined by extrapolating curves of the percentage of cells (from  
10 steps (d) and (f)) as a function of concentration (e.g., by testing only a few concentrations, such  
as 3 or fewer), or by empirically testing a full range of concentrations.

The above methods are useful for identifying an agent (test compound) that inhibits  
mitosis, for identifying a mitotic blocking agent which substantially retained its mitosis blocking  
effectiveness after its removal, and for predicting, for example, the  $IC_{50}$  or the  $IC_{95}$  of a mitotic  
15 blocking agent. When compared with relatively reversible antimitotic agents, substantially  
irreversible antimitotic agents, in other words, agents which continue to block mitosis in a cell  
which has been only transiently exposed to the agent, are likely to be more effective *in vivo*  
where natural processes, including multi-drug resistance (MDR) pumps and metabolic or other  
degradative pathways, prevent prolonged exposure. The effectiveness of relatively reversible  
20 antimitotic agents may depend upon a period of sustained exposure.

In view of the cost of developing pharmaceuticals, the economic advantages of  
determining reversibility ratios, as described above, are considerable. The above methods can be  
used, for example, to predict whether a test compound with good *in vitro* activity will be  
effective *in vivo*, such as in a clinical trial. Relatively reversible agents would not be expected to  
25 perform as well as irreversible agents. This is shown, for example, by contrasting the data for  
two known compounds, the relatively irreversible antimitotic agent vincristine and the highly  
reversible antimitotic agent vinblastine.

Table 2

**Reversibility Characteristics of Vinblastine and Vincristine**

+1310

Compound	Drug concentration required for complete mitotic block, nM		Reversibility ratio	Interpretation
	0 hour (before washout)	10 hour (after washout)		
Vinblastine	10	600	60	Highly Reversible
Vincristine	10	10	1	Irreversible

Analyses of the antimitotic drugs vinblastine and vincristine in the U937 Mitotic Block

5 Reversibility Assay indicate that despite identical potencies to induce initial mitotic blocks (0 hour values), the abilities of the two drugs to induce mitotic blocks which are sustained 10 hour after drug washout (10 hour values) are very different: vincristine induces irreversible mitotic blocks, while those induced by vinblastine are highly reversible.

Analyses of *in vivo* anticancer activities of the antimitotic drugs vinblastine and  
10 vincristine against COLO 205 human colon cancer xenografts grown sub-cutaneously in immunocompromised (nude) mice indicate that at equivalent doses of 1 mg/kg, vincristine shows substantial cancer growth inhibitory activity while vinblastine is inactive (FIG. 5). At the lower dose of 0.3 mg/kg, vincristine still produces moderate growth inhibition, while vinblastine is again inactive. The greater *in vivo* activity of vincristine correlates with its irreversibility relative  
15 to vinblastine's high reversibility.

#### E. Use

The disclosed compounds have pharmacological activity, including anti-tumor and anti-mitotic activity as demonstrated in section D above. Examples of tumors include melanoma, fibrosarcoma, monocytic leukemia, colon carcinoma, ovarian carcinoma, breast carcinoma, osteosarcoma, prostate carcinoma, lung carcinoma and ras-transformed fibroblasts.

The invention features pharmaceutical compositions which include a compound of formula (I) and a pharmaceutically-acceptable carrier. Compositions can also include a combination of disclosed compounds, or a combination of one or more disclosed compounds and other pharmaceutically-active agents, such as an anti-tumor agent, an immune-stimulating agent, an interferon, a cytokine, an anti-MDR agent or an anti-angiogenesis agent. Compositions can be formulated for oral, topical, parenteral, intravenous, or intramuscular administration, or administration by injection or inhalation. Formulations can also be prepared for controlled-release, including transdermal patches.

A method for inhibiting tumor growth in a patient includes the step of administering to the patient an effective, anti-tumor amount of a disclosed compound or composition. The invention also contemplates combination therapies, including methods of co-administering a compound of formula (I) before, during, or after administering another pharmaceutically active agent. The methods of administration may be the same or different. Inhibition of tumor growth includes a growth of the cell or tissue exposed to the test compound that is at least 20% less, and preferably 30%, 50%, or 75% less than the growth of the control (absence of known inhibitor or test compound).

#### Other Embodiments

The essential features of the invention can be easily discerned from the above description and the claims below. Based on the entire disclosure, variations of the disclosed compounds and methods of the invention described can be designed and adapted without departing from

the spirit and scope of the claims and the disclosure. References and publications described herein are hereby incorporated in their entirety.

What is claimed is: